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(54) **POLYNUCLEOTIDE ENCODING A MAIZE HERBICIDE RESISTANCE GENE AND METHODS FOR USE**

FÜR MAISHERBIZIDRESISTENZGEN KODIERENDES POLYNUKLEOTID UND
VERWENDUNGSVERFAHREN

POLYNUCLEOTIDE CODANT UN GENE DE RESISTANCE AUX DESHERBANTS DU MAIS ET
PROCEDES D'UTILISATION ASSOCIES

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Description**Field of the Invention**

[0001] This invention relates to compositions and methods useful in creating or enhancing herbicide resistance in plants. Additionally, the invention relates to plants that have been genetically transformed with the compositions of the invention.

Background of the invention

[0002] In the commercial production of crops, it is desirable to easily and quickly eliminate unwanted plants (*i.e.*, "weeds") from a field of crop plants. An ideal treatment would be one which could be applied to an entire field but which would eliminate only the unwanted plants while leaving the crop plants unharmed. One such treatment system involves the use of crop plants that are tolerant to a herbicide. When the herbicide is sprayed on a field of herbicide-tolerant crop plants, the crop plants continue to thrive while non-herbicide-tolerant weeds are killed or severely damaged.

[0003] Crop tolerance to specific herbicides can be conferred by engineering genes into crops which encode appropriate herbicide metabolizing enzymes. In some cases these enzymes, and the nucleic acids that encode them, originate in a plant. In other cases, they are derived from other organisms, such as microbes. See, *e.g.*, Padgett *et al.* (1996) "New weed control opportunities: Development of soybeans with a Round UP Ready™ gene" and Vasil (1996) "Phosphinothricin-resistant crops," both in *Herbicide-Resistant Crops*, ed. Duke (CRC Press, Boca Raton, Florida) pp.54-84 and pp. 85-91. Indeed, transgenic plants have been engineered to express a variety of herbicide tolerance genes from a variety of organisms, including a gene encoding a chimeric protein of rat cytochrome P4507A1 and yeast NADPH-cytochrome P450 oxidoreductase (Shiota *et al.* (1994) *Plant Physiol.* 106: 17), among other plant P450 genes (see, for example, Didierjean, L. *et al.* (2002) *Plant Physiol.* 130: 179-189; Morant, M.S. *et al.* (2003) *Opinion in Biotechnology* 14:151-162). Other genes that confer tolerance to herbicides include: acetohydroxy acid synthase ("AHAS"), which has been found to confer resistance to multiple types of ALS herbicides on plants expressing it and has been introduced into a variety of plants (see, *e.g.*, Hattori *et al.* (1995) *Mol. Gen. Genet.* 246: 419); glutathione reductase and superoxide dismutase (Aono *et al.* (1995) *Plant Cell Physiol.* 36: 1687); and genes for various phosphotransferases (Datta *et al.* (1992) *Plant Mol. Biol.* 20: 619).

[0004] While herbicide-tolerant crop plants are presently commercially available, improvements in every aspect of crop production are continuously in demand. Herbicides and crops that are presently commercially available unfortunately have particular characteristics which can limit their usefulness in commercial crop production. Particularly, individual herbicides have different and incomplete spectra of activity against common weed species.

[0005] The acetolactate synthase, or ALS (also known as AHAS) family of herbicides control weeds by inhibiting the production of branch chain of amino acids that are essential to plant growth and development. Specifically, they bind to the plant ALS enzyme. Commonly used herbicides in this family include nicosulfuron, rimsulfuron, and chlorsulfuron, among others. Herbicides in this category can be quite crop-specific. The invention relates to plants that are resistant to members of the ALS-inhibiting class of herbicides, which encompasses 5 sub-classes of herbicides including, but not limited to, the sulfonylurea (SU) family of herbicides and the imidazolinone family of herbicides.

[0006] The pigment synthesis-inhibiting class of herbicides targets the enzymes that allow plants to synthesize pigments, such as carotenoid pigments or chlorophyll pigments. Loss of pigment results in photo-destruction of chlorophyll and whitening of plant tissues, which is why these herbicides are often called "bleaching" herbicides. An example of a sub-class of the bleaching herbicides is the HPPD-inhibiting class, which inhibits the 4-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme (Lee *et al.* (1997) *Weed Sci.* 45:601-609). Herbicides in this family include, but are not limited to, mesotrione, tembotrione, topramezone and sulcotrione, among others. Corn is generally tolerant to mesotrione due to metabolism of the herbicide (Mitchell *et al.* (2001) *Pest Mgt. Sci.* 57:120-128). The same detoxification system may give tolerance to both mesotrione and some SU herbicides (Green & Williams (2004) *Proceedings Weed Science Society of America* 44:13). The invention relates to plants that are resistant to members of the pigment synthesis-inhibiting class of herbicides.

[0007] The protoporphyrinogen oxidase (PPO)-inhibiting class of herbicides interferes with the synthesis of chlorophyll, resulting in compounds that produce highly active compounds (free-radicals). These reactive compounds disrupt cell membranes which results in the leaf burning associated with post-emergence applications of these products. Herbicides in this family include, but are not limited to, acifluorfen, fomesafen, lactofen, sulfentrazone, carfentrazone, flumiclorac and flumioxazin, among others. The invention relates to plants that are resistant to members of the PPO-inhibiting class of herbicides.

[0008] Photosystem II (PSII)-inhibiting herbicides have a mode of action that involves interaction with components in the electron transfer chain of Photosystem II. Photosynthesis requires the transfer of electrons from Photosystem II to Photosystem I. A key step in this electron transfer chain is the reduction of plastoquinone (PQ) by the D₁ protein in the

thylakoid membrane. PSII-inhibitor herbicides bind to the D₁ protein, thus inhibiting PQ binding and interrupting the electron transfer process. This results in the plants not being able to fix carbon dioxide and produce the carbohydrates needed for the plant to survive. The block in electron transfer also causes an oxidative stress and the generation of radicals which cause rapid cellular damage. PSII-inhibiting herbicides are represented by several herbicide families, including the symmetrical triazines, triazinones (asymmetrical triazines), substituted ureas, uracils, pyridazinones, phenyl carbamates, nitriles, benzothiadiazoles, phenyl pyridazines, and acid amides. The invention relates to plants that are resistant to members of the PS II-inhibiting class of herbicides.

[0009] Synthetic auxin herbicides are a widely used class of herbicides that mimic the natural auxin hormones produced by plants. Auxins regulate many plant processes, including cell growth and differentiation. Auxins are generally present at low concentrations in the plant. Synthetic auxin herbicides mimic natural auxins and cause relatively high concentrations in the cell that result in a rapid growth response. Susceptible plants treated with these herbicides exhibit symptoms that could be called 'auxin overdose', and eventually die as a result of increased rates of disorganized and uncontrolled growth. The invention relates to plants that are resistant to members of the synthetic auxin class of herbicides.

[0010] This invention is based on the fine mapping, cloning and characterization of the gene responsible for an important herbicide resistance mechanism in maize.

[0011] It has been known since the early 1990s that natural tolerance in maize (*Zea mays* L.) to a subset of sulfonylurea herbicides (nicosulfuron [Dupont Accent® herbicide], rimsulfuron, primisulfuron, and thifensulfuron) is controlled by a single gene (named *nsf* by Kang (1993) Journal of Heredity 84(3): 216-217), with resistance dominant and sensitivity recessive (Harms et al. (1990) Theor. Appl. Genet. 80:353-358; Kang (1993) *supra*; Green & Uhrich (1993) Weed Sci. 41:508-516; Green & Uhrich (1994) Pestic. Sci. 40:187-191). It is also known that tolerant maize plants metabolize nicosulfuron by hydroxylation, with the characteristics of a cytochrome P450 (Fonne-Pfister et al. (1990) Pesticide Biochem. Physiol. 37:165-173; Brown & Cotterman (1994) Chem. Plant Prot. 10:47-81). It has been suggested that the same corn gene responsible for determining tolerance to some sulfonylurea herbicides is also responsible for the tolerance to bentazon (Barrett et al. (1997) Role of cytochrome P-450 in herbicide metabolism and selectivity and multiple herbicide metabolizing cytochrome P-450 activities in maize. In K. K. Hatzios, ed. Regulation of Enzymatic Systems Detoxifying Xenobiotics in Plants. Dordrecht: Kluwer Academic. pp. 35-50; Green (1998) Weed Technology 12:474-477) and HPPD inhibitor herbicides such as mesotrione (Green & Williams (2004) *supra*; Williams et al. (2005) HortScience 40(6):1801-1805). Recent advances in the development of the maize physical map and integrated markers (Bortiri et al. (2006) Curr Opin Plant Biol. 9(2):164-71) has allowed a positional cloning approach to be used for identifying the *Nsf1* locus.

[0012] The *Nsf1* resistance gene of the present invention encodes a novel gene related to the cytochrome P450 family. While multiple cytochrome P450 genes have been described, they differ widely in their response to different pathogens and exact action. The novel cytochrome P450 gene described in this disclosure has been demonstrated to provide improved tolerance or resistance to numerous herbicides, including nicosulfuron, rimsulfuron, primisulfuron, thifensulfuron and mesotrione.

Summary of the Invention

[0013] The present invention provides a use of a DNA construct comprising a polynucleotide comprising:

- (a) the nucleotide sequence of SEQ ID No. 1;
- (b) a nucleotide sequence encoding a polypeptide capable of conferring resistance to at least one herbicide to a plant, wherein said herbicide is a member of a class of herbicides selected from:

- (i) the ALS-inhibiting class;
- (ii) the pigment synthesis-inhibiting class;
- (iii) the PPO-inhibiting class;
- (iv) the PS II-inhibiting class; and
- (v) the synthetic auxin class

wherein the polypeptide has an amino acid sequence of at least 85%, at least 90%, or at least 95% identity, when compared to SEQ ID No. 2 based on the Needleman-Wunsch alignment algorithm, or

- (c) a complement of the nucleotide sequence of (a) or (b), wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary;

operably linked to at least one regulatory sequence;

to confer or enhance resistance to at least one herbicide to a plant, or to at least two, three, four or five herbicides, wherein said herbicides are selected from a class of herbicides selected from:

- (a) the ALS-inhibiting class;
- (b) the pigment synthesis-inhibiting class;
- (c) the PPO-inhibiting class;
- (d) the PS II-inhibiting class; and
- (e) the synthetic auxin class.

[0014] The present invention relates to a polynucleotide comprising a nucleotide sequence encoding a polypeptide capable of conferring resistance to at least one herbicide, wherein the polypeptide has an amino acid sequence of at least 85, 90 or 95% identity, when compared to SEQ ID NO: 2 based on the Needleman-Wunsch alignment algorithm, or a complement of the nucleotide sequence, wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary. The herbicides to which the polynucleotide of the embodiments imparts resistance include members of the ALS-inhibiting class; the pigment synthesis-inhibiting class; the PPO-inhibiting class; the PS II-inhibiting class; and the synthetic auxin class of herbicides. The polynucleotide of the embodiments may impart resistance to one or more herbicides from the same class, or from different classes, including representative members from all 5 classes.

[0015] The present invention also relates to uses of a vector comprising the polynucleotide and a recombinant DNA construct comprising the polynucleotide, operably linked to at least one regulatory sequence. A plant cell, as well as a plant and a seed each comprising the recombinant DNA construct of an embodiment of the present invention are also disclosed. Also contemplated are plants comprising additional polynucleotides encoding polypeptides responsible for traits of interest, such as polypeptides having glyphosate N-acetyltransferase activity, insecticidal Bt polypeptides, and other polypeptides of interest. Plants comprising these polynucleotides include monocots and dicots, including, but not limited to, maize, wheat, barley, oats, switchgrass, sorghum, rice, soybean, canola, potato, cotton, and sunflower.

[0016] The methods embodied by the present disclosure include 1) a method for transforming a cell, comprising transforming a cell with the polynucleotide of an embodiment of the present invention, 2) a method for producing a plant comprising transforming a plant cell with the recombinant DNA construct of an embodiment of the present invention and regenerating a plant from the transformed plant cell, and 3) methods of conferring or enhancing resistance to at least one herbicide, comprising transforming a plant with the recombinant DNA construct of an embodiment of the present invention, thereby conferring or enhancing resistance to at least one herbicide, such as a member of the ALS-inhibiting class; the pigment synthesis-inhibiting class; the PPO-inhibiting class; the PS II-inhibiting class; and the synthetic auxin class of herbicides.

[0017] In addition, the Examples below provide a variant allele of the *Nsf1* sequence in which a specific single amino acid change (see Example 2) renders the gene inoperative, resulting in sensitivity to at least one ALS or HPPD inhibitor herbicide to which most corn is resistant. This variant of the *Nsf1* gene can be used as a marker in breeding strategies to avoid incorporating the sensitive allele.

[0018] Methods of altering the level of expression of a protein capable of conferring resistance to at least one herbicide in a plant cell comprising (a) transforming a plant cell with the recombinant DNA construct of an embodiment of the present invention and (b) growing the transformed plant cell under conditions that are suitable for expression of the recombinant DNA construct wherein expression of the recombinant DNA construct results in production of altered levels of a protein capable of conferring resistance to at least one herbicide in the transformed host are disclosed. The herbicides for which resistance may be conferred include, for example, members of the ALS-inhibiting class; the pigment synthesis-inhibiting class; the PPO-inhibiting class; the PS II-inhibiting class; and the synthetic auxin class of herbicides.

[0019] Herbicides to which a polynucleotide of the invention may be used to confer or enhance resistance include, but are not limited to, herbicides selected from the ALS-inhibiting class of herbicides such as nicosulfuron, rimsulfuron, primisulfuron, imazethapyr, chlorsulfuron, chlorimuron ethyl, triasulfuron, flumetsulam and imazaquin. Additionally, such herbicides may be selected from the pigment synthesis-inhibiting class of herbicides, such as isoxaflutole, topramezone, sulcatrione and tembotrione. Such herbicides may also be selected from the PPO-inhibiting class of herbicides, such as acifluorfen, flumioxan and sulfentrazone. Optionally, such herbicides may be selected from the PS II-inhibiting class of herbicides, such as diuron, linuron, bentazon and chlorotoluron. Such herbicides may also be selected from the synthetic auxin class of herbicides, such as dicamba.

[0020] Methods of the disclosure include a method of determining the presence of the polynucleotide of the disclosure in a plant, comprising at least one of: (a) isolating nucleic acid molecules from the plant and determining if an *Nsf1* gene is present by attempting to amplify sequences homologous to the polynucleotide; or (b) isolating nucleic acid molecules from the plant and performing a Southern hybridization, or (c) isolating proteins from the plant and performing a western blot using antibodies to the NSF1 protein, or (d) isolating proteins from the plant and performing an ELISA assay using antibodies to the NSF1 protein, thereby determining the presence of the polynucleotide of the disclosure in the plant.

[0021] Plants with enhanced tolerance to at least one herbicide, comprising the *Nsf1* gene in a recombinant DNA construct, may further comprise a second herbicide resistance gene providing a certain level of tolerance to a herbicide selected from a class of herbicides selected from the group consisting of:

- (a) the ALS-inhibiting class;
- (b) the pigment synthesis-inhibiting class;
- (c) the PPO-inhibiting class;
- (d) the PS II-inhibiting class; and
- (e) the synthetic auxin class;

such that the presence of the *Nsf1* gene confers upon the plant a higher level of tolerance to the same herbicide than the tolerance level exhibited by a plant comprising the second herbicide resistance gene but not comprising the *Nsf1* gene.

[0022] Soybean plants comprising the *Nsf1* gene of the disclosure may also exhibit soybean cyst nematode resistance. Such plants may have been created through transformation or plant breeding techniques, and may have been bred from germplasm such as those selected from the group consisting of, Peking, PI88788, P189772, P190763, PI209332, P1404189A, PI437654, P1438489B, PI467312, PI468916, Hartwig, J87-233, and progeny derived from any of the listed sources.

Brief Description of the Drawings

[0023] Figure 1 (a-e) is a multiple sequence alignment of the polypeptide sequence of the embodiments (SEQ ID NO: 2) comparing it to other known Cytochrome P450 polypeptides (SEQ ID NOs: 3-13). Figure 1d also indicates the position of the most commonly conserved domain of the cytochrome P450 family (SEQ ID NO: 14). Identical residues in the alignment are indicated in upper case letters. Figure 2(a-b) is a multiple sequence alignment of the polypeptide sequences of several sensitive and resistant corn lines showing the commonly conserved domain of the cytochrome P450 family (SEQ ID NO: 14) as well as variations among the sequences.

Detailed Description of the Invention

[0024] The present disclosure relates to compositions and methods directed to inducing herbicide resistance in plants. The compositions are nucleotide and amino acid sequences that confer or enhance resistance to one or more members of one or more classes of herbicides, including the ALS-inhibiting, PPO-inhibiting, pigment synthesis-inhibiting, PS II-inhibiting and synthetic auxin herbicide classes, whose members include, but are not limited to, nicosulfuron, rimsulfuron, primisulfuron, and mesotrione. Specifically, certain polypeptides have the amino acid sequence set forth in SEQ ID NO: 2, and variants and fragments thereof. Nucleic acid molecules, and variants and fragments thereof, comprising nucleotide sequences that encode the amino acid sequence shown in SEQ ID NO: 2 may also be used in the invention.

[0025] One example of the native nucleotide sequence that encodes the polypeptide of SEQ ID NO: 2 is set forth in SEQ ID NO: 1. Plants, plant cells, seeds, and microorganisms comprising a nucleotide sequence that encodes a polypeptide of the embodiments are also disclosed herein.

[0026] The full length polypeptide (SEQ ID NO: 2) shares varying degrees of homology with known polypeptides of the cytochrome P450 family. In particular, the novel polypeptide of the disclosures shares homology with cytochrome P450 proteins isolated from *Oryza sativa*: Accession Nos. XP_469850 (SEQ ID NO: 3), ABC69856 (SEQ ID NO: 4); XP_469849 (SEQ ID NO: 11) and XP_469851 (SEQ ID NO: 12); and XP_469852 (SEQ ID NO: 13) and *Lolium rigidum*: Accession Nos. AAK38080 (SEQ ID NO: 5); AAK38079 (SEQ ID NO: 6); AAK38081 (SEQ ID NO: 7); BAD27508 (SEQ ID NO: 8); BAD27507 (SEQ ID NO: 9) and BAD27506 (SEQ ID NO: 10). Figure 1 provides an alignment of the amino acid sequence set forth in SEQ ID NO: 2 with the *O. sativa* and *L. rigidum* cytochrome P450 proteins (SEQ ID NOs: 3-13).

[0027] Amino acid alignments performed using the GAP program indicate that SEQ ID NO:2 shares the sequence similarities shown in Table 1 with the *O. sativa* and *L. rigidum* cytochrome P450 proteins.

Table 1: Comparison of NSF1 Peptide to other Cytochrome P450 peptides

Other Cytochrome P450 Protein	Percent Identity	Percent Similarity
XP_469850 (SEQ ID NO: 3)	67%	76%
ABC69856 (SEQ ID NO: 4)	67%	76%
AAK38080 (SEQ ID NO: 5)	68%	76%
AAK38079 (SEQ ID NO: 6)	67%	77%
AAK38081 (SEQ ID NO: 7)	67%	76%
BAD27508 (SEQ ID NO: 8)	67%	76%

(continued)

Other Cytochrome P450 Protein	Percent Identity	Percent Similarity
BAD27507 (SEQ ID NO: 9)	67%	76%
BAD27506 (SEQ ID NO: 10)	67%	76%
XP_469849 (SEQ ID NO: 11)	66%	75%
XP_469851 (SEQ ID NO: 12)	61%	71%
XP_469852 (SEQ ID NO: 13)	60%	72%

[0028] The cytochrome P450 family of genes in plants catalyze extremely diverse and often complex regiospecific and/or stereospecific reactions in the biosynthesis or catabolism of plant bioactive molecules. (Morant et al. (2003) Curr. Opin. Biotech. 14(2): 151-162). P450s are heme proteins that catalyze the activation of molecular oxygen by using electrons from NADPH. In the *Arabidopsis thaliana* genome alone, there are an estimated over 300 cytochromes P450 (Werck-Reichhart et al. (2000) Trends in Plant Science 5(3): 116-123). Common structural features occur in plant cytochromes P450 and help identify them as such. These features include the F-X-X-G-X-R-X-C-X-G (SEQ ID NO: 14) motif generally found near the C-terminus (see Figure 1d). About 150 residues upstream, another conserved motif generally found follows the A/G-G-X-D/E-T-T/S (SEQ ID NO: 15) motif and corresponds to the region of the peptide responsible for oxygen-binding and activation.

[0029] The nucleic acids and polypeptides disclosed herein find use in conferring or enhancing herbicide resistance to a plant. Accordingly, the compositions and methods disclosed herein are useful in protecting plants from damage caused by herbicides. "Herbicide resistance" is intended to mean that a plant or plant cell has the ability to tolerate a higher concentration of a herbicide than plants or cells which are not resistant, or to tolerate a certain concentration of a herbicide for a longer time than cells or plants which are not resistant. That is, herbicides are prevented from causing plant injury, or the injury caused by the herbicide is minimized or lessened, such as, for example, the reduction of leaf yellowing and associated yield loss. One of skill in the art will appreciate that the compositions and methods disclosed herein can be used with other compositions and methods available in the art for increasing or enhancing plant herbicide resistance. The term "enhance" refers to improve, increase, amplify, multiply, elevate, raise, and the like.

[0030] In particular aspects, the embodiments include use of a DNA construct, wherein the DNA construct comprises a nucleotide sequence encoding a herbicide resistance polypeptide of the embodiments operably linked to a promoter that drives expression in the plant for conferring or enhancing herbicide resistance in a plant. The plant expresses the polypeptide, thereby conferring or enhancing herbicide resistance upon the plant, or improving the plant's inherent level of resistance. The gene confers or enhances resistance to at least one herbicide of the ALS-inhibiting, pigment synthesis-inhibiting, PPO-inhibiting, PS II-inhibiting or synthetic auxin herbicide classes, whose members include, but are not limited to, the herbicides nicosulfuron, rimsulfuron, primisulfuron, thifensulfuron, bentazon, and mesotrione.

[0031] Expression of a polypeptide of the disclosure may be targeted to specific plant tissues, but generally in the case of herbicide resistance, continuous expression is desired throughout the cells of a plant. Therefore, while many promoters could be used in the embodiments of the invention, generally constitutive promoters are utilized. A constitutive promoter is a promoter that directs expression of a gene throughout the various parts of a plant and continuously throughout plant development.

[0032] As used herein, "nucleic acid" includes reference to a deoxyribonucleotide or ribonucleotide polymer. in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues (e.g., peptide nucleic acids) having the essential nature of natural nucleotides in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotides.

[0033] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. Polypeptides of the disclosures can be produced either from a nucleic acid disclosed herein, or by the use of standard molecular biology techniques. For example, a truncated protein of the disclosures can be produced by expression of a recombinant nucleic acid of the disclosures in an appropriate host cell, or alternatively by a combination of *ex vivo* procedures, such as protease digestion and purification.

[0034] As used herein, the terms "encoding" or "encoded" when used in the context of a specified nucleic acid mean that the nucleic acid comprises the requisite information to direct translation of the nucleotide sequence into a specified protein. The information by which a protein is encoded is specified by the use of codons. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid or may lack such intervening non-translated sequences (e.g., as in cDNA).

[0035] An "isolated" or "purified" polynucleotide or protein, or biologically active portion thereof, is substantially or essentially free from components that normally accompany or interact with the polynucleotide or protein as found in its naturally occurring environment. Thus, an isolated or purified polynucleotide or protein is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Optimally, an "isolated" polynucleotide is free of sequences (optimally protein encoding sequences) that naturally flank the polynucleotide (i.e., sequences located at the 5' and 3' ends of the polynucleotide) in the genomic DNA of the organism from which the polynucleotide is derived. For example, the isolated polynucleotide can contain less than about 5 kb, about 4 kb, about 3 kb, about 2 kb, about 1 kb, about 0.5 kb, or about 0.1 kb of nucleotide sequence that naturally flank the polynucleotide in genomic DNA of the cell from which the polynucleotide is derived. A protein that is substantially free of cellular material includes preparations of protein having less than about 30%, about 20%, about 10%, about 5%, or about 1 % (by dry weight) of contaminating protein. When the protein of the disclosures, or a biologically active portion thereof, is recombinantly produced, optimally culture medium represents less than about 30%, about 20%, about 10%, about 5%, or about 1% (by dry weight) of chemical precursors or non-protein-of-interest chemicals.

[0036] Fragments and variants of the disclosed nucleotide sequences and proteins encoded thereby are also encompassed. By "fragment" is intended a portion of the nucleotide sequence or a portion of the amino acid sequence and hence protein encoded thereby. Fragments of a nucleotide sequence encode protein fragments that retain the biological activity of the native protein and hence have the ability to confer or enhance resistance to at least one herbicide of the ALS-inhibiting, PPO-inhibiting, pigment synthesis-inhibiting, PS II-inhibiting or synthetic auxin herbicide class. Alternatively, fragments of a nucleotide sequence that are useful as hybridization probes do not necessarily encode fragment proteins retaining biological activity. Thus, fragments of a nucleotide sequence may range from at least about 15 nucleotides, about 50 nucleotides, about 100 nucleotides, and up to the full-length nucleotide sequence encoding the polypeptides of the disclosures.

[0037] A fragment of a nucleotide sequence that encodes a biologically active portion of a polypeptide of the embodiments will encode at least about 15, about 25, about 30, about 40, or about 50 contiguous amino acids, or up to the total number of amino acids present in a full-length polypeptide of the disclosures (for example, 521 amino acids for SEQ ID NO: 2). Fragments of a nucleotide sequence that are useful as hybridization probes or PCR primers generally need not encode a biologically active portion of a protein.

[0038] As used herein, "full-length sequence" in reference to a specified polynucleotide means having the entire nucleic acid sequence of a native sequence. By "native sequence" is intended an endogenous sequence, i.e., a non-engineered sequence found in an organism's genome.

[0039] Thus, a fragment of a nucleotide sequence may encode a biologically active portion of a polypeptide, or it may be a fragment that can be used as a hybridization probe or PCR primer using methods disclosed below. A biologically active portion of an herbicide resistance polypeptide can be prepared by isolating a portion of one of the nucleotide sequences of the embodiments, expressing the encoded portion of the protein and assessing the ability of the encoded portion of the protein to confer or enhance herbicide resistance in a plant. Nucleic acid molecules that are fragments of a nucleotide sequence of the embodiments comprise at least about 15, about 20, about 50, about 75, about 100, or about 150 nucleotides, or up to the number of nucleotides present in a full-length nucleotide sequence disclosed herein (for example, 1563 nucleotides for SEQ ID NO: 1).

[0040] "Variants" is intended to mean substantially similar sequences. For polynucleotides, a variant comprises a deletion and/or addition of one or more nucleotides at one or more internal sites within the native polynucleotide and/or a substitution of one or more nucleotides at one or more sites in the native polynucleotide. As used herein, a "native" polynucleotide or polypeptide comprises a naturally occurring nucleotide sequence or amino acid sequence, respectively. One of skill in the art will recognize that variants of the nucleic acids of the embodiments will be constructed such that the open reading frame is maintained. For polynucleotides, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of one of the polypeptides of the disclosure. Naturally occurring allelic variants such as these can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant polynucleotides also include synthetically derived polynucleotide, such as those generated, for example, by using site-directed mutagenesis but which still encode a protein of the disclosures. Generally, variants of a particular polynucleotide of the disclosures will have at least about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more sequence identity to that particular polynucleotide as determined by sequence alignment programs and parameters described elsewhere herein.

[0041] Variants of a particular polynucleotide (i.e., the reference polynucleotide) can also be evaluated by comparison of the percent sequence identity between the polypeptide encoded by a variant polynucleotide and the polypeptide encoded by the reference polynucleotide. Thus, for example, isolated polynucleotides that encode a polypeptide with a given percent sequence identity to the polypeptide of SEQ ID NO: 2 are disclosed. Percent sequence identity between

any two polypeptides can be calculated using sequence alignment programs and parameters described elsewhere herein. Where any given pair of polynucleotides of the embodiments is evaluated by comparison of the percent sequence identity shared by the two polypeptides they encode, the percent sequence identity between the two encoded polypeptides is at least about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91 %, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more sequence identity.

[0042] "Variant" protein is intended to mean a protein derived from the native protein by deletion or addition of one or more amino acids at one or more internal sites in the native protein and/or substitution of one or more amino acids at one or more sites in the native protein. Variant proteins are biologically active, that is they continue to possess the desired biological activity of the native protein, that is, the ability to confer or enhance plant herbicide resistance as described herein. Such variants may result from, for example, genetic polymorphism or from human manipulation. Biologically active variants of a native protein of the embodiments will have at least about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more sequence identity to the amino acid sequence for the native protein as determined by sequence alignment programs and parameters described elsewhere herein. A biologically active variant of a protein may differ from that protein by as few as about 1-15 amino acid residues, as few as about 1-10, such as about 6-10, as few as about 5, as few as 4, 3, 2, or even 1 amino acid residue.

[0043] The proteins of the disclosures may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants and fragments of the herbicide resistance proteins can be prepared by mutations in the DNA. Methods for mutagenesis and polynucleotide alterations are well known in the art. See, for example, Kunkel (1985) Proc. Natl. Acad. Sci. USA 82:488-492; Kunkel et al. (1987) Methods in Enzymol. 154:367-382; U.S. Patent No. 4,873,192; Walker and Gaastra, eds. (1983) Techniques in Molecular Biology (MacMillan Publishing Company, New York) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff et al. (1978) Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D.C.). Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be optimal.

[0044] Thus, the genes and polynucleotides of the disclosures include both the naturally occurring sequences as well as mutant forms. Likewise, the proteins of the embodiments encompass both naturally occurring proteins as well as variations and modified forms thereof. Such variants will continue to possess the desired ability to confer or enhance plant resistance to at least one herbicide of the ALS-inhibiting, PPO-inhibiting, pigment synthesis-inhibiting, PS II-inhibiting or synthetic auxin herbicide classes. Obviously, the mutations that will be made in the DNA encoding the variant must not place the sequence out of reading frame and optimally will not create complementary regions that could produce secondary mRNA structure. See, EP Patent No. 0075444.

[0045] The deletions, insertions, and substitutions of the protein sequences disclosed herein are not expected to produce radical changes in the characteristics of the protein. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by screening transgenic plants which have been transformed with the variant protein to ascertain the effect on the herbicide resistance characteristics of the plant.

[0046] Variant polynucleotides and proteins also encompass sequences and proteins derived from mutagenic or recombinogenic procedures, including and not limited to procedures such as DNA shuffling. One of skill in the art could envision modifications that would alter the range of herbicides to which the protein responds. With such a procedure, one or more different protein coding sequences can be manipulated to create a new protein possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. For example, using this approach, sequence motifs encoding a domain of interest may be shuffled between the protein gene of the embodiments and other known protein genes to obtain a new gene coding for a protein with an improved property of interest, such as increased ability to confer or enhance plant herbicide resistance. Strategies for such DNA shuffling are known in the art. See, for example, US 2002/0058249; Stemmer (1994) Proc. Natl. Acad. Sci. USA 91:10747-10751; Stemmer (1994) Nature 370:389-391; Crameri et al. (1997) Nature Biotech. 15:436-438; Moore et al. (1997) J. Mol. Biol. 272:336-347; Zhang et al. (1997) Proc. Natl. Acad. Sci. USA 94:4504-4509; Crameri et al. (1998) Nature 391:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458.

The polynucleotides of the disclosure can be used to isolate corresponding sequences from other organisms, particularly other plants. In this manner, methods such as PCR, hybridization, and the like can be used to identify such sequences based on their sequence homology to the sequences set forth herein. Sequences isolated based on their sequence identity to the entire sequences set forth herein or to variants and fragments thereof are encompassed by the disclosures. Such sequences include sequences that are orthologs of the disclosed sequences. "Orthologs" is intended to mean genes derived from a common ancestral gene and which are found in different species as a result of speciation. Genes

found in different species are considered orthologs when their nucleotide sequences and/or their encoded protein sequences share at least about 60%, about 70%, about 75%, about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or greater sequence identity. Functions of orthologs are often highly conserved among species. Thus, isolated polynucleotides that encode for a protein that confers or enhances plant herbicide resistance and that hybridize under stringent conditions to the sequences disclosed herein can be used in the invention.

[0047] In a PCR approach, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any organism of interest. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York). See also Innis et al., eds. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York); Innis and Gelfand, eds. (1995) *PCR Strategies* (Academic Press, New York); and Innis and Gelfand, eds. (1999) *PCR Methods Manual* (Academic Press, New York). Known methods of PCR include, and are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like.

[0048] In hybridization techniques, all or part of a known polynucleotide is used as a probe that selectively hybridizes to other corresponding polynucleotides present in a population of cloned genomic DNA fragments or cDNA fragments (i.e., genomic or cDNA libraries) from a chosen organism. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ³²P, or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the polynucleotides of the embodiments. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook et al. (1989) *supra*.

[0049] For example, an entire polynucleotide disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding polynucleotides and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique and are optimally at least about 10 nucleotides in length, at least about 15 nucleotides in length, or at least about 20 nucleotides in length. Such probes may be used to amplify corresponding polynucleotides from a chosen organism by PCR. This technique may be used to isolate additional coding sequences from a desired organism or as a diagnostic assay to determine the presence of coding sequences in an organism. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook et al. (1989) *supra*).

[0050] Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, optimally less than 500 nucleotides in length.

[0051] Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30 °C for short probes (e.g., 10 to 50 nucleotides) and at least about 60 °C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37 °C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55 °C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37 °C, and a wash in 0.5X to 1X SSC at 55 to 60 °C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37 °C, and a final wash in 0.1X SSC at 60 to 65 °C for at least 30 minutes. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours. The duration of the wash time will be at least a length of time sufficient to reach equilibrium.

[0052] Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the thermal melting point (T_m) can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: $T_m = 81.5^\circ\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\% \text{ form}) - 500/L$; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1 °C for each 1% of mismatching;

thus, T_m , hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq 90\%$ identity are sought, the T_m can be decreased 10 °C. Generally, stringent conditions are selected to be about 5 °C lower than the T_m for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4 °C lower than the T_m ; moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10 °C lower than the T_m ; low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20 °C lower than the T_m . Using the equation, hybridization and wash compositions, and desired T_m , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45 °C (aqueous solution) or 32 °C (formamide solution), it is optimal to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Ausubel et al., eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al.* (1989) *supra*.

[0053] Various procedures can be used to check for the presence or absence of a particular sequence of DNA, RNA, or a protein. These include, for example, Southern blots, northern blots, western blots, and ELISA analysis. Techniques such as these are well known to those of skill in the art and many references exist which provide detailed protocols. Such references include Sambrook *et al.* (1989) *supra*, and Crowther, J.R. (2001), *The ELISA Guidebook*, Humana Press, Totowa, NJ, USA.

[0054] The following terms are used to describe the sequence relationships between two or more polynucleotides or polypeptides: (a) "reference sequence," (b) "comparison window," (c) "sequence identity," and, (d) "percentage of sequence identity."

(a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two polynucleotides. Generally, the comparison window is at least about 20 contiguous nucleotides in length, and optionally can be about 30, about 40, about 50, about 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

[0055] Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent sequence identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local alignment algorithm of Smith et al. (1981) *Adv. Appl. Math.* 2:482; the global alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453; the search-for-local alignment method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

[0056] Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, and are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the GCG Wisconsin Genetics Software Package, Version 10 (available from Accelrys Inc., 9685 Scranton Road, San Diego, California, USA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins et al. (1988) *Gene* 73:237-244 (1988); Higgins et al. (1989) *CABIOS* 5:151-153; Corpet et al. (1988) *Nucleic Acids Res.* 16:10881-90; Huang et al. (1992) *CABIOS* 8:155-65; and Pearson et al. (1994) *Meth. Mol. Biol.* 24:307-331. The ALIGN program is based on the algorithm of Myers and Miller (1988) *supra*. A PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used with the ALIGN program when comparing amino acid sequences. The BLAST programs of Altschul et al (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) *supra*. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleotide sequence encoding a protein of the disclosures. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a protein or polypeptide of the embodiments. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra*. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g., BLASTN

for nucleotide sequences, BLASTX for proteins) can be used. See www.ncbi.nlm.nih.gov. Alignment may also be performed manually by inspection.

[0057] Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using Gap Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using Gap Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

[0058] GAP uses the algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443-453, to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of matched bases and the fewest gaps. It allows for the provision of a gap creation penalty and a gap extension penalty in units of matched bases. GAP must make a profit of gap creation penalty number of matches for each gap it inserts. If a gap extension penalty greater than zero is chosen, GAP must, in addition, make a profit for each gap inserted of the length of the gap times the gap extension penalty. Default gap creation penalty values and gap extension penalty values in Version 10 of the GCG Wisconsin Genetics Software Package for protein sequences are 8 and 2, respectively. For nucleotide sequences the default gap creation penalty is 50 while the default gap extension penalty is 3. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 200. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or greater.

[0059] GAP presents one member of the family of best alignments. There may be many members of this family, and no other member has a better quality. GAP displays four figures of merit for alignments: Quality, Ratio, Identity, and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the GCG Wisconsin Genetics Software Package is BLOSUM62 (see Henikoff and Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

(c) As used herein, "sequence identity" or "identity" in the context of two polynucleotides or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity." Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

[0060] The use of the term "polynucleotide" is not intended to be limited to polynucleotides comprising DNA. Those of ordinary skill in the art will recognize that polynucleotides can comprise ribonucleotides and combinations of ribonucleotides and deoxyribonucleotides. Such deoxyribonucleotides and ribonucleotides include both naturally occurring molecules and synthetic analogues. The polynucleotides of the disclosures also encompass all forms of sequences including, and not limited to, single-stranded forms, double-stranded forms, and the like.

[0061] Isolated polynucleotides of the present disclosures can be incorporated into recombinant DNA constructs ca-

pable of introduction into and replication in a host cell. A "vector" may be such a construct that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels et al., *Cloning Vectors: A Laboratory Manual*, 1985, supp. 1987; Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989; and Flevin et al., *Plant Molecular Biology Manual*, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a signal peptide sequence for targeted expression, a transcription termination site, and/or a polyadenylation signal.

[0062] The terms "recombinant construct," "expression cassette," "expression construct," "chimeric construct," "construct," "recombinant DNA construct," "DNA construct" and "recombinant DNA fragment" are used interchangeably herein and are nucleic acid fragments. A recombinant construct comprises an artificial combination of nucleic acid fragments, including, and not limited to, regulatory and coding sequences that are not found together in nature. For example, a recombinant DNA construct may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source and arranged in a manner different than that found in nature. Such construct may be used by itself or may be used in conjunction with a vector. If a vector is used then the choice of vector is dependent upon the method that will be used to transform host cells as is well known to those skilled in the art. For example, a plasmid vector can be used. The skilled artisan is well aware of the genetic elements that must be present on the vector in order to successfully transform, select and propagate host cells comprising any of the isolated nucleic acid fragments of the invention. Screening to obtain lines displaying the desired expression level and pattern of the polynucleotides or of the *Nsf1* locus may be accomplished by amplification, Southern analysis of DNA, Northern analysis of mRNA expression, immunoblotting analysis of protein expression, phenotypic analysis, and the like.

[0063] The term "recombinant DNA construct" refers to a DNA construct assembled from nucleic acid fragments obtained from different sources. The types and origins of the nucleic acid fragments may be very diverse.

[0064] In some embodiments, DNA constructs comprising a promoter operably linked to a heterologous nucleotide sequence of the embodiments are further disclosed. The DNA constructs of the embodiments find use in generating transformed plants, plant cells, and microorganisms and in practicing the methods for inducing ALS and HPPD inhibitor herbicide resistance disclosed herein. The DNA construct will include 5' and 3' regulatory sequences operably linked to a polynucleotide of the embodiments. "Operably linked" is intended to mean a functional linkage between two or more elements. "Regulatory sequences" refer to nucleotides located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which may influence the transcription, RNA processing, stability, or translation of the associated coding sequence. Regulatory sequences may include, and are not limited to, promoters, translation leader sequences, introns, and polyadenylation recognition sequences. For example, an operable linkage between a polynucleotide of interest and a regulatory sequence (a promoter, for example) is functional link that allows for expression of the polynucleotide of interest. Operably linked elements may be contiguous or non-contiguous. When used to refer to the joining of two protein coding regions, operably linked is intended to mean that the coding regions are in the same reading frame. The coding sequence may additionally contain a sequence used to target the protein to the chloroplast, the vacuole, the endoplasmic reticulum or to the outside of the cell. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple DNA constructs. Such a DNA construct is provided with a plurality of restriction sites and/or recombination sites for insertion of the polynucleotide that encodes a herbicide resistance polypeptide to be under the transcriptional regulation of the regulatory regions. The DNA construct may additionally contain selectable marker genes.

[0065] The DNA construct will include in the 5'-3' direction of transcription, a transcriptional initiation region (i.e., a promoter), translational initiation region, a polynucleotide of the embodiments, a translational termination region and, optionally, a transcriptional termination region functional in the host organism. The regulatory regions (i.e., promoters, transcriptional regulatory regions, and translational termination regions) and/or the polynucleotide of the disclosures may be native/analogous to the host cell or to each other. Alternatively, the regulatory regions and/or the polynucleotide of the embodiments may be heterologous to the host cell or to each other. As used herein, "heterologous" in reference to a sequence is a sequence that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous polynucleotide is from a species different from the species from which the polynucleotide was derived, or, if from the same/analogous species, one or both are substantially modified from their original form and/or genomic locus, or the promoter is not the native promoter for the operably linked polynucleotide.

[0066] The optionally included termination region may be native with the transcriptional initiation region, may be native with the operably linked polynucleotide of interest, may be native with the plant host, or may be derived from another

source (i.e., foreign or heterologous) to the promoter, the polynucleotide of interest, the host, or any combination thereof. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau et al. (1991) Mol. Gen. Genet. 262:141-144; Proudfoot (1991) Cell 64:671-674; Sanfacon et al. (1991) Genes Dev. 5:141-149; Mogen et al. (1990) Plant Cell 2:1261-1272; Munroe et al. (1990) Gene 91:151-158; Ballas et al. (1989) Nucleic Acids Res. 17:7891-7903; and Joshi et al. (1987) Nucleic Acids Res. 15:9627-9639. In particular embodiments, the potato protease inhibitor II gene (PinII) terminator is used. See, for example, Keil et al. (1986) Nucl. Acids Res. 14:5641-5650; and An et al. (1989) Plant Cell 1:115-122.

[0067] A number of promoters can be used in the practice of the embodiments, including the native promoter of the polynucleotide sequence of interest. The promoters can be selected based on the desired outcome. A wide range of plant promoters are discussed in the recent review of Potenza et al. (2004) In Vitro Cell Dev Biol - Plant 40:1-22. For example, the nucleic acids can be combined with constitutive, tissue-preferred, pathogen-inducible, or other promoters for expression in plants. Such constitutive promoters include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Patent No. 6,072,050; the core CaMV 35S promoter (Odell et al. (1985) Nature 313:810-812); rice actin (McElroy et al. (1990) Plant Cell 2:163-171); ubiquitin (Christensen et al. (1989) Plant Mol. Biol. 12:619-632 and Christensen et al. (1992) Plant Mol. Biol. 18:675-689); pEMU (Last et al. (1991) Theor. Appl. Genet. 81:581-588); MAS (Velten et al. (1984) EMBO J. 3:2723-2730); ALS promoter (U.S. Patent No. 5,659,026), and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611.

[0068] Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

[0069] DNA constructs may additionally contain 5' leader sequences. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein et al. (1989) Proc. Natl. Acad. Sci. USA 86:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Gallie et al. (1995) Gene 165(2):233-238), MDMV leader (Maize Dwarf Mosaic Virus), and human immunoglobulin heavy-chain binding protein (BiP) (Macejak et al. (1991) Nature 353:90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling et al. (1987) Nature 325:622-625); tobacco mosaic virus leader (TMV) (Gallie et al. (1989) in Molecular Biology of RNA, ed. Cech (Liss, New York), pp. 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel et al. (1991) Virology 81:382-385). See also, Della-Cioppa et al. (1987) Plant Physiol. 84:965-968. Other methods known to enhance translation can also be utilized, for example, introns, and the like.

[0070] In preparing the DNA construct, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

[0071] The DNA construct can also comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). Additional selectable markers include phenotypic markers such as β -galactosidase and fluorescent proteins such as green fluorescent protein (GFP) (Su et al. (2004) Biotechnol Bioeng 85:610-9 and Fetter et al. (2004) Plant Cell 16:215-28), cyan fluorescent protein (CYP) (Bolte et al. (2004) J. Cell Science 117:943-54 and Kato et al. (2002) Plant Physiol 129:913-42), and yellow fluorescent protein (PhiYFP™ from Evrogen, see, Bottle et al. (2004) J. Cell Science 117:943-54). For additional selectable markers, see generally, Yarranton (1992) Curr. Opin. Biotech. 3:506-511; Christopherson et al. (1992) Proc. Natl. Acad. Sci. USA 89:6314-6318; Yao et al. (1992) Cell 71:63-72; Reznikoff (1992) Mol. Microbiol. 6:2419-2422; Barkley et al. (1980) in The Operon, pp. 177-220; Hu et al. (1987) Cell 48:555-566; Brown et al. (1987) Cell 49:603-612; Figge et al. (1988) Cell 52: 713-722; Deuschle et al. (1989) Proc. Natl. Acad. Sci. USA 86:5400-5404; Fuerst et al. (1989) Proc. Natl. Acad. Sci. USA 86:2549-2553; Deuschle et al. (1990) Science 248:480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines et al. (1993) Proc. Natl. Acad. Sci. USA 90:1917-1921; Labow et al. (1990) Mol. Cell. Biol. 10:3343-3356; Zambretti et al. (1992) Proc. Natl. Acad. Sci. USA 89:3952-3956; Baim et al. (1991) Proc. Natl. Acad. Sci. USA 88:5072-5076; Wyborski et al. (1991) Nucleic Acids Res. 19:4647-4653; Hillenand-Wissman (1989) Topics Mol. Struc. Biol. 10:143-162; Degenkolb et al. (1991) Antimicrob. Agents Chemother. 35:1591-1595; Kleinschmidt et al. (1988) Biochemistry 27:1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen et al. (1992) Proc. Natl. Acad. Sci. USA 89:5547-5551; Oliva et al. (1992)

Antimicrob. Agents Chemother. 36:913-919; Hlavka et al. (1985) Handbook of Experimental Pharmacology, Vol. 78 (Springer-Verlag, Berlin); Gill et al. (1988) Nature 334:721-724.

[0072] The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used.

[0073] The gene of the disclosures can be expressed as a transgene in order to make plants resistant to at least one herbicide of the ALS-inhibiting, PPO-inhibiting, pigment synthesis-inhibiting, PS II-inhibiting or synthetic auxin herbicide classes. Using the different promoters described elsewhere in this disclosure, this will allow its expression in a modulated form in different circumstances. One can also insert the entire gene, both native promoter and coding sequence, as a transgene. Finally, using the gene of the embodiments as a transgene will allow quick combination with other traits, such as insect or fungal resistance.

[0074] In certain embodiments the nucleic acid sequences of the disclosures can be stacked with any combination of polynucleotide sequences of interest, which may be transgenic or non-transgenic, in order to create plants with a desired phenotype. For example, the polynucleotides of the disclosures may be stacked with any other polynucleotides of the disclosures, or with other genes. The combinations generated can also include multiple copies of any one of the polynucleotides of interest. The polynucleotides of the disclosures can also be stacked with any other gene or combination of genes to produce plants with a variety of desired trait combinations including and not limited to traits desirable for animal feed such as high oil genes (e.g., U.S. Patent No. 6,232,529); balanced amino acids (e.g. hordothionins (U.S. Patent Nos. 5,990,389; 5,885,801; 5,885,802; and 5,703,409); barley high lysine (Williamson et al. (1987) Eur. J. Biochem. 165:99-106; and WO 98/20122); and high methionine proteins (Pedersen et al. (1986) J. Biol. Chem. 261:6279; Kirihaara et al. (1988) Gene 71:359; and Musumura et al. (1989) Plant Mol. Biol. 12: 123)); increased digestibility (e.g., modified storage proteins (U.S. Application Serial No. 10/053,410, filed November 7, 2001); and thioredoxins (U.S. Application Serial No. 10/005,429, filed December 3, 2001)). The polynucleotides of the disclosures can also be stacked with traits desirable for insect, disease or herbicide resistance (e.g., *Bacillus thuringiensis* toxin proteins (U.S. Patent Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; Geiser et al (1986) Gene 48:109); lectins (Van Damme et al. (1994) Plant Mol. Biol. 24:825); fumonisin detoxification genes (U.S. Patent No. 5,792,931); avirulence and disease resistance genes (Jones et al. (1994) Science 266:789; Martin et al. (1993) Science 262:1432; Mindrinos et al. (1994) Cell 78:1089); acetolactate synthase (ALS) mutants that lead to herbicide resistance such as the S4 and/or Hra mutations (Lee et al., (1988) EMBO J. 7(5):1241-1248), resistance to inhibitors of glutamine synthase such as phosphinothricin or basta (e.g., bar gene; De Block et al. (1987) EMBO J. 6:2513-2518); HPPD genes that confer tolerance to HPPD inhibiting herbicides such as mesotrione or isoxaflutole (Matringe et al. (2005) Pest Management Science 61:269-276; Dufourmantel et al., (2007) Plant Biotech. J. 5:118-133; see also WO1997049816), genes for tolerance to PPO inhibiting herbicides (Li and Nicholl (2005) Pest Management Science 61:277-285); synthetic auxin resistance genes (US patent application 2005/014737 and Herman et al., (2005) J. Biol. Chem. 280: 24759-24767), and glyphosate resistance (epsps genes, *gat* genes such as those disclosed in U.S. Patent Application Publication US2004/0082770, also WO02/36782 and WO03/092360)); and traits desirable for processing or process products such as high oil (e.g., U.S. Patent No. 6,232,529); modified oils (e.g., fatty acid desaturase genes (U.S. Patent No. 5,952,544; WO 94/11516)); modified starches (e.g., ADPG pyrophosphorylases (AGPase), starch synthases (SS), starch branching enzymes (SBE) and starch debranching enzymes (SDBE)); and polymers or bioplastics (e.g., U.S. patent No. 5,602,321; beta-ketothiolase, polyhydroxybutyrate synthase, and acetoacetyl-CoA reductase (Schubert et al. (1988) J. Bacteriol. 170:5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs)). One could also combine the polynucleotides of the disclosures with polynucleotides providing agronomic traits such as male sterility (e.g., see U.S. Patent No. 5,583,210), stalk strength, flowering time, yield improvement, or transformation technology traits such as cell cycle regulation or gene targeting (e.g. WO 99/61619; WO 00/17364; WO 99/25821).

[0075] These stacked combinations can be created by any method including and not limited to cross breeding plants by any conventional or TopCross® methodology, or genetic transformation. If the traits are stacked by genetically transforming the plants, the polynucleotide sequences of interest can be combined at any time and in any order. For example, a transgenic plant comprising one or more desired traits can be used as the target to introduce further traits by subsequent transformation. The traits can be introduced simultaneously in a co-transformation protocol with the polynucleotides of interest provided by any combination of transformation cassettes. For example, if two sequences will be introduced, the two sequences can be contained in separate transformation cassettes (trans) or contained on the same transformation cassette (cis). Expression of the sequences can be driven by the same promoter or by different promoters. In certain cases, it may be desirable to introduce a transformation cassette that will suppress the expression of the polynucleotide of interest. This may be combined with any combination of other suppression cassettes or overexpression cassettes to generate the desired combination of traits in the plant. It is further recognized that polynucleotide sequences can be stacked at a desired genomic location using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840, WO99/25855, and WO99/25853. Further disclosures include plants obtainable by a method comprising: crossing a plant containing the *Nsf1* gene as a first parent plant, with a different plant that lacks an *Nsf1* gene as a second parent plant, thereby to obtain progeny comprising the *Nsf1* gene of the first parent; and optionally further comprising one or more further breeding steps to obtain progeny of one or more further generations comprising

the *Nsf1* gene of the first parent. Such disclosed plants can include both inbred and hybrid plants. Seeds of such plants, including those seeds which are homozygous and heterozygous for the *Nsf1* gene, and methods of obtaining plant products resulting from the processing of those seeds are embodied in the disclosure. Using such seed in food or feed or the production of a corn product, such as flour, meal and oil is also an embodiment of the disclosure.

[0076] An "ancestral line" or "progenitor" is a parent line used as a source of genes, e.g., for the development of elite lines. "Progeny" are the descendents of the ancestral line, and may be separated from their ancestors by many generations of breeding. An "elite line" or "elite variety" is an agronomically superior line or variety that has resulted from many cycles of breeding and selection for superior agronomic performance. Similarly, "elite germplasm" is an agronomically superior germplasm, typically derived from and/or capable of giving rise to a plant with superior agronomic performance, such as an existing or newly developed elite line of corn or soybeans.

[0077] Also embodied in the disclosure is the use of molecular markers to move the gene or transgene into elite lines using breeding techniques. Molecular markers can be used in a variety of plant breeding applications (eg see Staub et al. (1996) Hortscience 31: 729-741; Tanksley (1983) Plant Molecular Biology Reporter. 1: 3-8). One of the main areas of interest is to increase the efficiency of backcrossing and introgressing genes using marker-assisted selection (MAS). A molecular marker that demonstrates linkage with a locus affecting a desired phenotypic trait provides a useful tool for the selection of the trait in a plant population. This is particularly true where the phenotype is hard to assay, e.g. many disease resistance traits, or, occurs at a late stage in the plants development, e.g. seed characteristics. Since DNA marker assays are less laborious, and take up less physical space, than field phenotyping, much larger populations can be assayed, increasing the chances of finding a recombinant with the target segment from the donor line moved to the recipient line. The closer the linkage, the more useful the marker, as recombination is less likely to occur between the marker and the gene causing the trait, which can result in false positives. Having flanking markers decreases the chances that false positive selection will occur as a double recombination event would be needed. The ideal situation is to have a marker in the gene itself, so that recombination can not occur between the marker and the gene. Such a marker is called a 'perfect marker'.

[0078] Optionally, the nucleic acids may be targeted to the chloroplast for expression. In this manner, where the nucleic acid is not directly inserted into the chloroplasts. Such transit peptides are known in the art. See, for example, Von Heijne et al. (1991) Plant Mol. Biol. Rep. 9:104-126; Clark et al. (1989) J. Biol. Chem. 264:17544-17550; Della-Cioppa et al. (1987) Plant Physiol. 84:965-968; Romer et al. (1993) Biochem. Biophys. Res. Commun. 196:1414-1421; and Shah et al. (1986) Science 233:478-481.

[0079] Chloroplast targeting sequences are known in the art and include the chloroplast small subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco) (de Castro Silva Filho et al. (1996) Plant Mol. Biol. 30:769-780; Schnell et al. (1991) J. Biol. Chem. 266(5):3335-3342); 5-(enolpyruvyl)shikimate-3-phosphate synthase (EPSPS) (Archer et al. (1990) J. Bioenerg. Biomemb. 22(6):789-810); tryptophan synthase (Zhao et al. (1995) J. Biol. Chem. 270(11):6081-6087); plastocyanin (Lawrence et al. (1997) J. Biol. Chem. 272(33):20357-20363); chorismate synthase (Schmidt et al. (1993) J. Biol. Chem. 268(36):27447-27457); and the light harvesting chlorophyll a/b binding protein (LHBP) (Lamppa et al. (1988) J. Biol. Chem. 263:14996-14999). See also Von Heijne et al. (1991) Plant Mol. Biol. Rep. 9:104-126; Clark et al. (1989) J. Biol. Chem. 264:17544-17550; Della-Cioppa et al. (1987) Plant Physiol. 84:965-968; Romer et al. (1993) Biochem. Biophys. Res. Commun. 196:1414-1421; and Shah et al. (1986) Science 233:478-481.

[0080] Methods for transformation of chloroplasts are known in the art. See, for example, Svab et al. (1990) Proc. Natl. Acad. Sci. USA 87:8526-8530; Svab and Maliga (1993) Proc. Natl. Acad. Sci. USA 90:913-917; Svab and Maliga (1993) EMBO J. 12:601-606. The method relies on particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination. Additionally, plastid transformation can be accomplished by transactivation of a silent plastid-borne transgene by tissue-preferred expression of a nuclear-encoded and plastid-directed RNA polymerase. Such a system has been reported in McBride et al. (1994) Proc. Natl. Acad. Sci. USA 91:7301-7305.

[0081] The nucleic acids to be targeted to the chloroplast may be optimized for expression in the chloroplast to account for differences in codon usage between the plant nucleus and this organelle. In this manner, the nucleic acids of interest may be synthesized using chloroplast-preferred codons. See, for example, U.S. Patent No. 5,380,831.

[0082] The uses of the embodiments may involve, and are not limited to, introducing a polypeptide or polynucleotide into a plant. "Introducing" is intended to mean presenting to the plant the polynucleotide. In some embodiments, the polynucleotide will be presented in such a manner that the sequence gains access to the interior of a cell of the plant, including its potential insertion into the genome of a plant. The uses of the embodiments do not depend on a particular method for introducing a sequence into a plant, only that the polynucleotide gains access to the interior of at least one cell of the plant. Methods for introducing polynucleotides into plants are known in the art including, and not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

[0083] "Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. "Host cell" refers the cell into which transformation of the recombinant DNA construct takes

place and may include a yeast cell, a bacterial cell, and a plant cell. Examples of methods of plant transformation include *Agrobacterium-mediated* transformation (De Blaere et al., 1987, Meth. Enzymol. 143:277) and particle-accelerated or "gene gun" transformation technology (Klein et al., 1987, Nature (London) 327:70-73; U.S. Patent No. 4,945,050), among others.

[0084] "Stable transformation" is intended to mean that the nucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by the progeny thereof. "Transient transformation" or "transient expression" is intended to mean that a polynucleotide is introduced into the plant and does not integrate into the genome of the plant or a polypeptide is introduced into a plant.

[0085] Transformation protocols as well as protocols for introducing polypeptides or polynucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of introducing polypeptides and polynucleotides into plant cells include microinjection (Crossway et al. (1986) Biotechniques 4:320-334), electroporation (Riggs et al. (1986) Proc. Natl. Acad. Sci. USA 83:5602-5606, *Agrobacterium-mediated* transformation (U.S. Patent Nos. 5,563,055-and 5,981,840), direct gene transfer (Paszkowski et al. (1984) EMBO J. 3:2717-2722), and ballistic particle acceleration (see, for example, Sanford et al., U.S. Patent Nos. 4,945,050; 5,879,918; 5,886,244; and 5,932,782; Tomes et al. (1995) in Plant Cell, Tissue, and Organ Culture: Fundamental Methods, ed. Gamborg and Phillips (Springer-Verlag, Berlin); McCabe et al. (1988) Biotechnology 6:923-926); and Lec1 transformation (WO 00/28058). Also see Weissinger et al. (1988) Ann. Rev. Genet. 22:421-477; Sanford et al. (1987) Particulate Science and Technology 5:27-37 (onion); Christou et al. (1988) Plant Physiol. 87:671-674 (soybean); McCabe et al. (1988) Bio/Technology 6:923-926 (soybean); Finer and McMullen (1991) In Vitro Cell Dev. Biol. 27P:175-182 (soybean); Singh et al. (1998) Theor. Appl. Genet. 96:319-324 (soybean); Datta et al. (1990) Biotechnology 8:736-740 (rice); Klein et al. (1988) Proc. Natl. Acad. Sci. USA 85:4305-4309 (maize); Klein et al. (1988) Biotechnology 6:559-563 (maize); U.S. Patent Nos. 5,240,855; 5,322,783 and 5,324,646; Klein et al. (1988) Plant Physiol. 91:440-444 (maize); Fromm et al. (1990) Biotechnology 8:833-839 (maize); Hooykaas-Van Slogteren et al. (1984) Nature (London) 311:763-764; U.S. Patent No. 5,736,369 (cereals); Bytebier et al. (1987) Proc. Natl. Acad. Sci. USA 84:5345-5349 (Liliaceae); De Wet et al. (1985) in The Experimental Manipulation of Ovule Tissues, ed. Chapman et al. (Longman, New York), pp. 197-209 (pollen); Kaeppler et al. (1990) Plant Cell Reports 9:415-418 and Kaeppler et al. (1992) Theor. Appl. Genet. 84:560-566 (whisker-mediated transformation); D'Halluin et al. (1992) Plant Cell 4:1495-1505 (electroporation); Li et al. (1993) Plant Cell Reports 12:250-255 and Christou and Ford (1995) Annals of Botany 75:407-413 (rice); Osjoda et al. (1996) Nature Biotechnology 14:745-750 (maize via *Agrobacterium tumefaciens*). Methods are known in the art for the targeted insertion of a polynucleotide at a specific location in the plant genome. In one embodiment, the insertion of the polynucleotide at a desired genomic location is achieved using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840, WO99/25855, and WO99/25853. Briefly, the polynucleotide of the disclosures can be contained in transfer cassette flanked by two non-identical recombination sites. The transfer cassette is introduced into a plant have stably incorporated into its genome a target site which is flanked by two non-identical recombination sites that correspond to the sites of the transfer cassette. An appropriate recombinase is provided and the transfer cassette is integrated at the target site. The polynucleotide of interest is thereby integrated at a specific chromosomal position in the plant genome.

[0086] The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick et al. (1986) Plant Cell Reports 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting progeny having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved. In this manner, the embodiments provides transformed seed (also referred to as "transgenic seed") having a nucleotide construct of the embodiments, for example, a DNA construct of the disclosures, stably incorporated into their genome.

[0087] As used herein, the term plant includes plant cells, plant protoplasts, plant cell tissue cultures from which maize plant can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants such as embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit, kernels, ears, cobs, husks, stalks, roots, root tips, anthers, and the like. Grain is intended to mean the mature seed produced by commercial growers for purposes other than growing or reproducing the species. Progeny, variants, and mutants of the regenerated plants are also included within the scope of the disclosures, provided that these parts comprise the introduced polynucleotides.

[0088] The invention may be used to confer or enhance herbicide resistance in plants, especially soy (*Glycine max*). Other plant species may also be of interest in practicing the embodiments of the invention, including, and not limited to, other dicot and monocot crop plants. The maize gene of the embodiments is commonly found in the majority of commercial corn lines, most of which are naturally tolerant to at least one, and usually several, synthetic auxin, ALS-, PS II- and pigment synthesis-inhibitor herbicides, such as rimsulfuron, nicosulfuron and mesotrione.

[0089] It is therefore envisioned that the same tolerance to certain herbicides present in most corn lines can be extended to other crop plants by transgenic means though the use of the endogenous maize *Nsf1* gene and variants thereof.

Listings of maize lines with tolerance or sensitivity to selected SU herbicides are widely available, such as those provided by the USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Network - (GRIN)*. [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland. [retrieved on March 6, 2006]: Retrieved from the internet: <URL: http://www.ars-grin.gov/cgi-bin/npgs/html/dno_eval_acc.pl?89201+153002+21 > ; and the "Maize Germplasm Lines" listings available from the Buckler Laboratory website [retrieved on March 6, 2006]: Retrieved from the internet: <URL: <http://www.maizegenetics.net/index.php?page=germplasm/lines.html>>, and also in reference articles such as Kang (1993) J. Heredity. 84(3): 216-217.

[0090] Where appropriate, the polynucleotides may be optimized for increased expression in the transformed organism. For example, the polynucleotides can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell and Gowri (1990) Plant Physiol. 92:1-11 for a discussion of host-preferred codon usage. Methods are available in the art for synthesizing plant-preferred genes. See, for example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray et al. (1989) Nucleic Acids Res. 17:477-498.

[0091] The present invention may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plants of interest include, but are not limited to, corn (*Zea mays*), *Brassica* sp. (e.g., *B. napus*, *B. rapa*, *B. juncea*), alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), millet (e.g., pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense*, *Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats (*Avena* spp.), barley, palm, coconut, castor bean, olive, beans (for example guar, locust bean, fenugreek, soybean, garden beans, mung beans, lima beans, fava beans), peas (such as cowpeas, field peas, lentils, chickpeas, etc.), vegetables, ornamentals, and conifers.

[0092] Other plants of interest for the invention include those which have the potential for use as biofuel crops, including, but not limited to, prairie grasses such as switchgrass (*Panicum virgatum*), elephant grass (*Pennisetum purpureum*), Johnson grass (*Sorghum halepense*), *Miscanthus* spp., as well as hybrid poplar and hybrid willow trees.

[0093] Vegetables include tomatoes (*Lycopersicon esculentum*), lettuce (e.g., *Lactuca sativa*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus* spp.), and members of the genus *Cucumis* such as cucumber (*C. sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*). Ornamentals include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia (*Euphorbia pulcherrima*), and chrysanthemum.

[0094] The disclosure provides not only a gene for use in transgenic applications, but sequences and methods that allow the resistance gene to be used as a marker in corn breeding strategies. For example, the gene of the disclosures, or the locus containing it, may be identified in a crop line intended to be used for breeding. Breeders would generally want to avoid using crop lines that are sensitive to herbicides where there is usually natural tolerance. Accordingly, the identification of the sequence of the *Nsf1* gene will help breeders to identify and avoid creating herbicide-sensitive lines.

[0095] Nucleic acid based markers can be developed and applied using many different technologies. Such technologies include, and are not limited to, Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeat (SSR), Random Amplified Polymorphic DNA (RAPD), Cleaved Amplified Polymorphic Sequences (CAPS) (Rafalski and Tingey, 1993, Trends in Genetics 9:275-280), Amplified Fragment Length Polymorphism (AFLP) (Vos et al., 1995, Nucleic Acids Res. 23:4407-4414), Single Nucleotide Polymorphism (SNP) (Brookes, 1999, Gene 234:177-186), Sequence Characterized Amplified Region (SCAR) (Paran and Michelmore, 1993, Theor. Appl. Genet. 85:985-993), Sequence Tagged Site (STS) (Onozaki et al., 2004, Euphytica 138:255-262), Single Stranded Conformation Polymorphism (SSCP) (Orita et al., 1989, Proc Natl Acad Sci USA 86:2766-2770), Inter-Simple Sequence Repeat (ISSR) (Blair et al., 1999, Theor. Appl. Genet. 98:780-792), Inter-Retrotransposon Amplified Polymorphism (IRAP), Retrotransposon-Microsatellite Amplified Polymorphism (REMAP) (Kalendar et al. (1999) Theor. Appl. Genet. 98:704-711) and the like.

[0096] As used herein, "locus" shall refer to a genetically defined region of a chromosome carrying a gene or, possibly, two or more genes so closely linked that genetically they behave as a single locus, responsible for a phenotype. A "gene" shall refer to a specific gene within that locus, including its associated regulatory sequences. Thus, the *Nsf1* locus refers to the chromosomal region genetically defined as conferring resistance to at least one herbicide of the ALS-inhibiting, PPO-inhibiting, pigment synthesis-inhibiting, PS II-inhibiting and synthetic auxin herbicide class. One embodiment of the present disclosure is the isolation of the *Nsf1* gene and the demonstration that it is the gene responsible for the phenotype conferred by the presence of the locus. Genetically defined loci are by their nature not as precisely defined in terms of size as genes, which can be delineated molecularly.

[0097] Units, prefixes, and symbols may be denoted in their SI accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range. Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. The above-defined terms are more fully defined by reference to the specification as a whole.

Examples

[0098] The embodiments of the invention are further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of the embodiments of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications to adapt it to various usages and conditions. Thus, various modifications of the embodiments of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

Example 1

Identification of the *Nsf1* Gene through Positional Cloning

[0099] A BC1 population (expected 50% *Nsf1/insf1*, 50% *nsf1/insf1*) was developed using the sensitive inbred W703A as the recurrent parent, and either B73 or Q66 as the resistant line. Plants were misted with a 2.3 mM nicosulfuron, 0.5% v/v Kinetic surfactant solution at approximately the V3 stage. Both resistant and sensitive parents were also grown and sprayed as controls. In order to avoid falsely classifying a plant which may have died due to reasons other than the herbicide application, only resistant progeny were sampled and analyzed. A total of 96 resistant plants were used for the initial mapping. This was sufficient to place *Nsf1* between markers umc1766 and umc2036, and thus on contig 202 of the maize B73-based physical map ((Retrieved on March 6, 2006) Retrieved from the internet <URL: http://www.gramene.org/Zea_mays/cytoview?contig=ctg202&x=44&y=9>).

[0100] Based on BAC-end sequences of a maize Mo17-based contig, flanking CAPS (cleaved amplified polymorphic sequence) markers were identified on BACs of contig 202.

[0101] For finer mapping of this interval, a total of 388 resistant plants were used in the next step. Based on sequencing of subcloned fragments of BACs in this interval, two flanking CAPs markers were found on overlapping BACs. Both of these markers had 2/388 recombinants.

[0102] Both of these BACs were sequenced and analyzed. Within the 163 kb region of the 2 BACs flanked by two proprietary markers, P1 and P2, there were several putative genes. For the third round of mapping, a total of 2584 resistant plants were used, and markers were developed to separate some of the genes. One marker showed 11/2584 (0.4%) recombinants, helping to eliminate certain genes as being responsible for the resistance. Two other markers each had 2 (0.08%) recombinants, eliminating yet another gene. Finally, a marker between two genes had a single recombinant (0.04%), eliminating one of those two genes. Thus it was determined which gene was the gene of interest. The gene, *Nsf1*, was determined to have homology to some cytochrome P450 genes known in the art.

Example 2

Analysis of the *Nsf1* Gene

[0103] Analysis of the Gene 18 (*Nsf1*) sequence in the B73-derived BAC shows an open reading frame of 521 amino acids, and containing the conserved heme-binding motif FXXGXXXCXG (SEQ ID NO: 14) found in all cytochrome P450s (Figures 1d and 2b).

[0104] In order to determine if the *Nsf1* allele was consistent across maize lines, three corn lines with unknown sensitivity levels to nicosulfuron were tested to determine their reaction and then evaluate their sequences. Plants were misted with a 2.3 mM nicosulfuron, 0.5% v/v Kinetic surfactant solution at approximately the V3 stage. Both known resistant and sensitive lines were also grown and sprayed as controls. Results of the testing of the three lines showed that lines Q66 and Black Mexican Sweet (BMS) were resistant and line A188 was sensitive.

[0105] Of these two other resistant lines, Q66 and BMS, also possess this ORF, although Q66 differs from both B73 and BMS by 3 amino acids (Figure 2a and 2b) These three variant amino acids are marked with bold type and rectangles in Figures 2a and 2b in the Q66 sequence string to show their positions. Analysis of a sensitive line, GA209, shows an

insertion of 392 bp relative to the resistant lines which results in a frameshift and an open reading frame of only 338 amino acids (Figure 2b). A survey of numerous North American sensitive lines showed that many of the sensitive lines contain this same insertion of unknown DNA.

[0106] Analysis of the sequence from the F2 sensitive line showed that there is only one nucleotide difference between B73 (SEQ ID NO: 2) and F2 (SEQ ID NO: 22), which changes amino acid 263 from arginine to threonine (Figure 2b). This single change therefore eliminates the resistance phenotype and variant sequences with such a change are expected not to retain biological activity. This change is useful in developing an SNP to assist corn breeders in avoiding the susceptible allele.

[0107] *Nsf1* is 67% identical to a rice cytochrome P450 which has recently been reported to control sulfonylurea sensitivity in that plant (Accession No: ABC69856, SEQ ID NO: 4).

[0108] Genomic sequence from B73 shows a single intron with the expected GT left border and AG right border. The position of the intron is shown in the sequence listing in SEQ ID NO: 16.

[0109] The cloning of this gene has a number of potential applications. It could be used as a selectable marker for transformation in a sensitive transformable line such as A188 (Ishida et al., (1996) Nature Biotechnology 14:745-750). A transgene designed to suppress the *Nsf1* gene function would function as a dominant negative selectable marker. *Nsf1* could also be used to create transgenic resistance in other plants, such as soybean, which are sensitive to this subclass of sulfonylureas.

Example 3:

Testing of Maize Plants for Sensitivity to Nicosulfuron

[0110] Three corn lines with unknown sensitivity levels to nicosulfuron were tested to determine their reaction. Plants were misted with a 2.3 mM nicosulfuron, 0.5% v/v Kinetic surfactant solution at approximately the V3 stage. Both known resistant and sensitive lines were also grown and sprayed as controls. Results of the testing of the three lines showed that lines Q66 and BMS were resistant and line A188 was sensitive.

Example 4: Preparation of Transgenic Soybean Plants

[0111] The following stock solutions and media were used for transformation and regeneration of soybean plants:

Stock solutions

Sulfate 100 X Stock: 37.0 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.69 g $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.86 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0025 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$.

Halides 100 X Stock: 30.0 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.083 g KI, 0.0025 g $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$,

P, B, Mo 100X Stock: 18.5 g KH_2PO_4 , 0.62 g H_3BO_3 , 0.025 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$

Fe EDTA 100X Stock: 3.724 g Na_2EDTA , 2.784 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$.

2,4-D Stock: 10 mg/mL.

Vitamin B5 1000X Stock: 10.0 g *myo*-inositol, 0.10 g nicotinic acid, 0.10 g pyridoxine HCl, 1 g thiamine.

Media (per Liter)

SB196: 10 mL of each of the above stock solutions, 1 mL B5 Vitamin stock, 0.463 g $(\text{NH}_4)_2\text{SO}_4$, 2.83 g KNO_3 , 1 mL 2,4-D stock, 1 g asparagine, 10 g sucrose, pH 5.7.

SB103: 1 pk. Murashige & Skoog salts mixture, 1 mL B5 Vitamin stock, 750 mg MgCl_2 hexahydrate, 60 g maltose, 2 g gelrite, pH 5.7.

SB166: SB103 supplemented with 5 g per liter activated charcoal.

SB71-4: Gamborg's B5 salts (Gibco-BRL catalog No. 21153-028), 1 mL B5 vitamin stock, 30 g sucrose, 5 g TC agar, pH 5.7.

[0112] Soybean embryogenic suspension cultures were maintained in 35 mL liquid medium (SB196) on a rotary shaker (150 rpm) at 28 °C with fluorescent lights providing a 16-hour day/8-hour night cycle. Cultures were subcultured every 2 weeks by inoculating approximately 35 mg of tissue into 35 mL of fresh liquid media.

[0113] Soybean embryogenic suspension cultures were transformed by particle gun bombardment (see Klein et al. (1987) Nature 327:70-73) using a DuPont Biolistic PDS1000/He instrument.

[0114] The recombinant DNA plasmid used to express *Nsf1* was on a separate recombinant DNA plasmid from the selectable marker gene. Both recombinant DNA plasmids were co-precipitated onto gold particles as follows. The DNAs in suspension were added to 50 μL of a 20 - 60 mg/mL 0.6 μm gold particle suspension and then combined with 50 μL

CaCl₂ (2.5 M) and 20 µL spermidine (0.1 M). The mixture was pulse vortexed 5 times, spun in a microfuge for 10 seconds, and the supernatant removed. The DNA-coated particles are then washed once with 150 µL of 100% ethanol, pulse vortexed and spun in a microfuge again, and resuspended in 85 µL of anhydrous ethanol. Five µL of the DNA-coated gold particles are then loaded on each macrocarrier disk.

[0115] Approximately 150 to 250 mg of two-week-old suspension culture was placed in an empty 60 mm x 15 mm petri plate and the residual liquid is removed from the tissue using a pipette. The tissue was placed about 3.5 inches away from a retaining screen and each plate of tissue was bombarded once. Membrane rupture pressure was set at 650 psi and the chamber was evacuated to - 28 inches of Hg. Eighteen plates were bombarded, and, following bombardment, the tissue from each plate was divided between two flasks, placed back into liquid media, and cultured as described above.

[0116] Seven days after bombardment, the liquid medium was exchanged with fresh SB196 medium supplemented with 50 mg/mL hygromycin. The selective medium was refreshed weekly or biweekly. Seven weeks post-bombardment, green, transformed tissue was observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue was removed and inoculated into individual flasks to generate new, clonally-propagated, transformed embryogenic suspension cultures. Thus, each new line was treated as an independent transformation event. These suspensions were then maintained as suspensions of embryos clustered in an immature developmental stage through subculture or were regenerated into whole plants by maturation and germination of individual somatic embryos.

[0117] Transformed embryogenic clusters were removed from liquid culture and placed on solid agar medium (SB166) containing no hormones or antibiotics for one week. Embryos were cultured at 26 °C with mixed fluorescent and incandescent lights on a 16-hour day: 8-hour night schedule. After one week, the cultures were then transferred to SB103 medium and maintained in the same growth conditions for 3 additional weeks. Prior to transfer from liquid culture to solid medium, tissue from selected lines was assayed by PCR for the presence of the chimeric gene. Somatic embryos became suitable for germination after 4 weeks and were then removed from the maturation medium and dried in empty petri dishes for one to five days. The dried embryos were then planted in SB71-4 medium and allowed to germinate under the same light and germination conditions described above. Germinated embryos were transferred to sterile soil and grown to maturity.

Example 5

T0 and T1 Transgenic Plant Analysis

T0 Testing

[0118] Two different constructs comprising the *Nsf1* gene were created to examine herbicide efficacy of the gene when transformed into soybean. The *Nsf1* constructs were co-bombarded with a 35S:HYG insert to permit event selection using hygromycin.

[0119] At the V2 to V6 growth stage, a total of 127 T0 plants were sprayed with 35 g/ha rimsulfuron. All rimsulfuron treatments were applied with 0.2% w/w nonionic surfactant in a spray volume of 287 L/ha. In addition to the T0 plants, replications of three different controls were included - two positive and one negative. Individual plants were evaluated for herbicide response at ten days after treatment, and assigned a visual response score from 1 to 9 (1 = dead plant to 9 = no effect observed). Based upon high tolerance scores to the initial rimsulfuron spray, five T0 events were sprayed with an additional 35 g/ha rimsulfuron. Plants were rated for visual tolerance using a 1 to 9 score at ten days after the second application.

[0120] In the T0 generation, 4 of 51 events had improved tolerance compared to the controls at ten days after treatment with 35 g/ha rimsulfuron. Three of 51 T0 events had improved level of tolerance after an additional application of 35 g/ha rimsulfuron. Two of these 51 events were advanced to the T1 generation for more extensive herbicide testing.

T1 Testing

[0121] Two events from the T0 generation were advanced to the T1 generation for additional herbicide efficacy testing of the *Nsf1* gene. Replicates of two controls, as well as T1 plants, were grown in greenhouse experiments and sprayed with mesotrione at one of two rates (200 g/ha or 50 g/ha), nicosulfuron (70 g/ha), or rimsulfuron (35 g/ha) at the V3 growth stage. All herbicide treatments were applied with 1 % w/w modified seed oil adjuvant in a spray volume of 374 L/ha. Plants were rated for herbicide response at eight days after application using a 1 to 9 score as used in the T0 testing.

[0122] An expanded herbicide efficacy test was developed in a second T1 plant experiment for the same two events advanced from the T0 generation. At the V3 growth stage, plants were sprayed with different treatments of herbicides that would typically cause substantial crop injury when applied to commodity soybean at the rates examined. All herbicide treatments were applied in a spray volume of 287 L/ha. Isoxaflutole (140 g/ha), topramezone (140 g/ha), and sulcotrione

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(140 g/ha) were applied with 1% w/w modified seed oil adjuvant. Diuron treatments (560 g/ha) were applied with 1% w/w petroleum crop oil adjuvant. Acifluorfen (4480 g/ha), sulfentrazone (140 g/ha), flumioxazin (140 g/ha), and dicamba (280 g/ha) were applied with 0.25% w/w nonionic surfactant. Rimsulfuron (35 g/ha) treatments were applied with 0.5% w/w basic blend adjuvant. At eight and fifteen days after treatment, plants were rated visually for crop injury using a 0 to 100 scale (0 = no injury to 100 = dead plant). Since the T1 events were segregating, only the plants with the best overall scores were selected, corresponding to the 75% that would be expected to possess the transgene.

[0123] One of the two events had significantly better tolerance compared to the controls at 8 DAT and 15 DAT after application of acifluorfen, dicamba, diuron, flumioxazin, isoxaflutole, mesotrione, rimsulfuron, sulcotrione, sulfentrazone, and topramezone treatments. The second event had significantly better tolerance compared to the controls at 15 DAT after application of acifluorfen, dicamba, isoxaflutole, mesotrione, rimsulfuron, sulcotrione, sulfentrazone, and topramezone treatments. Although the exact expression level of the *Nsf1* gene in the events tested was not determined, transgenic soybean plants comprising the maize *Nsf1* gene displayed better tolerance to a range of different herbicides when compared directly to control plants.

SEQUENCE LISTING

[0124]

<110> E.I. du Pont de Nemours and Company

<120> Polynucleotide encoding a maize herbicide resistance gene and methods for use

<130> 2068-PCT

<150> 60/780,946

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<150> 60/888,634

<151> 2007-02-07

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<220>

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	1				5					10					15		
5	ctc	cac	tac	ctc	ctg	ggc	cgg	cgg	gcc	ggc	ggc	gag	ggc	aag	gcc	aag	96
	Leu	His	Tyr	Leu	Leu	Gly	Arg	Arg	Ala	Gly	Gly	Glu	Gly	Lys	Ala	Lys	
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10	gcc	aag	ggc	tcg	cgg	cgg	cgg	ctc	ccg	ccg	agc	cct	ccg	gcg	atc	ccg	144
	Ala	Lys	Gly	Ser	Arg	Arg	Arg	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Ile	Pro	
			35					40					45				
15	ttc	ctg	ggc	cac	ctc	cac	ctc	gtc	aag	gcc	ccg	ttc	cac	ggg	gcg	ctg	192
	Phe	Leu	Gly	His	Leu	His	Leu	Val	Lys	Ala	Pro	Phe	His	Gly	Ala	Leu	
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20	gcc	cgc	ctc	gcg	gcg	cgc	cac	ggc	ccg	gtg	ttc	tcc	atg	cgc	ctg	ggg	240
	Ala	Arg	Leu	Ala	Ala	Arg	His	Gly	Pro	Val	Phe	Ser	Met	Arg	Leu	Gly	
	65					70					75					80	
25	acc	cgg	cgc	gcc	gtg	gtc	gtg	tcg	tcg	ccg	gac	tgc	gcc	agg	gag	tgc	288
	Thr	Arg	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Asp	Cys	Ala	Arg	Glu	Cys	
					85					90					95		
30	ttc	acg	gag	cac	gac	gtg	aac	ttc	gcg	aac	cgg	ccg	ctg	ttc	ccg	tcg	336
	Phe	Thr	Glu	His	Asp	Val	Asn	Phe	Ala	Asn	Arg	Pro	Leu	Phe	Pro	Ser	
				100					105					110			

30

35

40

45

50

55

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	Gly	Pro	Tyr	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Ala	Val	Gln	Leu	Leu	
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	Arg	Ala	Met	Val	Arg	Arg	Met	Asp	Arg	Ala	Ala	Ala	Ala	Gly	Gly	Gly	
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20	ggc	gtc	gcg	cgc	gtc	cag	ctc	aag	cgg	cgg	ctg	ttc	gag	ctc	tcc	ctc	576
	Gly	Val	Ala	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	
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	Ser	Val	Leu	Met	Glu	Thr	Ile	Ala	His	Thr	Lys	Thr	Ser	Arg	Ala	Glu	
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	Ala	Asp	Ala	Asp	Ser	Asp	Met	Ser	Thr	Glu	Ala	His	Glu	Phe	Lys	Gln	
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	Tyr	Leu	Pro	Val	Leu	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys	
				245				250						255			
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	Ile	Leu	Asp	Ala	Val	Gly	Arg	Arg	Asp	Ala	Phe	Leu	Gly	Arg	Leu	Ile	
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	Asp	Gly	Glu	Arg	Arg	Arg	Leu	Asp	Ala	Gly	Asp	Glu	Ser	Glu	Ser	Lys	
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	Ser	Met	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Ser	Glu	Pro	Glu	Val	
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	Tyr	Thr	Asp	Thr	Val	Ile	Thr	Ala	Leu	Cys	Ala	Asn	Leu	Phe	Gly	Ala	
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	Gly	Thr	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu	
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	Thr Tyr Leu Gln Cys Ile Val Asp Glu Thr Leu Arg Leu His Pro Ala	
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10	gcg ccg ctg ctg ctg ccg cac gag tcc gcc gcg gac tgc acg gtc ggc	1200
	Ala Pro Leu Leu Leu Pro His Glu Ser Ala Ala Asp Cys Thr Val Gly	
	385 390 395 400	
15	ggc tac gac gtc ccg cgc ggc acg atg ctg ctg gtc aac gtc cac gcg	1248
	Gly Tyr Asp Val Pro Arg Gly Thr Met Leu Leu Val Asn Val His Ala	
	405 410 415	
20	gtc cac agg gac ccc gcg gtc tgg gag gac ccg gac agg ttc gtc ccg	1296
	Val His Arg Asp Pro Ala Val Trp Glu Asp Pro Asp Arg Phe Val Pro	
	420 425 430	
25	gag cgg ttc gag ggc gcc ggc ggc aag gcc gag ggg cgc ctg ctg atg	1344
	Glu Arg Phe Glu Gly Ala Gly Gly Lys Ala Glu Gly Arg Leu Leu Met	
	435 440 445	
30	ccg ttc ggg atg ggg cgg cgc aag tgc ccc ggg gag acg ctc gcg ctg	1392
	Pro Phe Gly Met Gly Arg Arg Lys Cys Pro Gly Glu Thr Leu Ala Leu	
	450 455 460	
35	cgg acc gtc ggg ctg gtc ctc gcc acg ctg ctc cag tgc ttc gac tgg	1440
	Arg Thr Val Gly Leu Val Leu Ala Thr Leu Leu Gln Cys Phe Asp Trp	
	465 470 475 480	
40	gac acg gtt gat gga gct cag gtt gac atg aag gct agc ggc ggg ctg	1488
	Asp Thr Val Asp Gly Ala Gln Val Asp Met Lys Ala Ser Gly Gly Leu	
	485 490 495	
45	acc atg ccc cgg gcc gtc ccg ttg gag gcc atg tgc agg ccg cgt aca	1536
	Thr Met Pro Arg Ala Val Pro Leu Glu Ala Met Cys Arg Pro Arg Thr	
	500 505 510	
50	gct atg cgt ggt gtt ctt aag agg ctc	1563
	Ala Met Arg Gly Val Leu Lys Arg Leu	
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	<213> Zea mays	
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65	Leu His Tyr Leu Leu Gly Arg Arg Ala Gly Gly Glu Gly Lys Ala Lys	
	20 25 30	
70	Ala Lys Gly Ser Arg Arg Arg Leu Pro Pro Ser Pro Pro Ala Ile Pro	
	35 40 45	
75	Phe Leu Gly His Leu His Leu Val Lys Ala Pro Phe His Gly Ala Leu	
	50 55 60	
80	Ala Arg Leu Ala Ala Arg His Gly Pro Val Phe Ser Met Arg Leu Gly	
	65 70 75 80	

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	Thr	Arg	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Asp	Cys	Ala	Arg	Glu	Cys
					85					90					95	
	Phe	Thr	Glu	His	Asp	Val	Asn	Phe	Ala	Asn	Arg	Pro	Leu	Phe	Pro	Ser
				100					105					110		
5	Met	Arg	Leu	Ala	Ser	Phe	Asp	Gly	Ala	Met	Leu	Ser	Val	Ser	Ser	Tyr
			115					120					125			
	Gly	Pro	Tyr	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Ala	Val	Gln	Leu	Leu
		130					135					140				
	Ser	Ala	His	Arg	Val	Gly	Cys	Met	Ala	Pro	Ala	Ile	Glu	Ala	Gln	Val
10		145				150					155					160
	Arg	Ala	Met	Val	Arg	Arg	Met	Asp	Arg	Ala	Ala	Ala	Ala	Gly	Gly	Gly
				165						170					175	
	Gly	Val	Ala	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu
				180					185					190		
15	Ser	Val	Leu	Met	Glu	Thr	Ile	Ala	His	Thr	Lys	Thr	Ser	Arg	Ala	Glu
			195					200					205			
	Ala	Asp	Ala	Asp	Ser	Asp	Met	Ser	Thr	Glu	Ala	His	Glu	Phe	Lys	Gln
		210					215					220				
	Ile	Val	Asp	Glu	Leu	Val	Pro	Tyr	Ile	Gly	Thr	Ala	Asn	Arg	Trp	Asp
		225				230					235					240
20	Tyr	Leu	Pro	Val	Leu	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys
				245						250					255	
	Ile	Leu	Asp	Ala	Val	Gly	Arg	Arg	Asp	Ala	Phe	Leu	Gly	Arg	Leu	Ile
				260					265					270		
	Asp	Gly	Glu	Arg	Arg	Arg	Leu	Asp	Ala	Gly	Asp	Glu	Ser	Glu	Ser	Lys
			275					280					285			
25	Ser	Met	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Ser	Glu	Pro	Glu	Val
		290					295					300				
	Tyr	Thr	Asp	Thr	Val	Ile	Thr	Ala	Leu	Cys	Ala	Asn	Leu	Phe	Gly	Ala
					310						315					320
	Gly	Thr	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu
30					325					330					335	
	Leu	Asn	His	Arg	Glu	Ala	Leu	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala
				340					345					350		
	Ala	Val	Gly	Thr	Ser	Arg	Leu	Val	Thr	Ala	Asp	Asp	Val	Pro	His	Leu
			355					360					365			
35	Thr	Tyr	Leu	Gln	Cys	Ile	Val	Asp	Glu	Thr	Leu	Arg	Leu	His	Pro	Ala
		370					375					380				
	Ala	Pro	Leu	Leu	Leu	Pro	His	Glu	Ser	Ala	Ala	Asp	Cys	Thr	Val	Gly
					390						395					400
	Gly	Tyr	Asp	Val	Pro	Arg	Gly	Thr	Met	Leu	Leu	Val	Asn	Val	His	Ala
				405						410					415	
40	Val	His	Arg	Asp	Pro	Ala	Val	Trp	Glu	Asp	Pro	Asp	Arg	Phe	Val	Pro
				420					425					430		
	Glu	Arg	Phe	Glu	Gly	Ala	Gly	Gly	Lys	Ala	Glu	Gly	Arg	Leu	Leu	Met
			435					440					445			
	Pro	Phe	Gly	Met	Gly	Arg	Arg	Lys	Cys	Pro	Gly	Glu	Thr	Leu	Ala	Leu
		450					455					460				
45	Arg	Thr	Val	Gly	Leu	Val	Leu	Ala	Thr	Leu	Leu	Gln	Cys	Phe	Asp	Trp
					470							475				480
	Asp	Thr	Val	Asp	Gly	Ala	Gln	Val	Asp	Met	Lys	Ala	Ser	Gly	Gly	Leu
					485					490					495	
	Thr	Met	Pro	Arg	Ala	Val	Pro	Leu	Glu	Ala	Met	Cys	Arg	Pro	Arg	Thr
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	Ala	Met	Arg	Gly	Val	Leu	Lys	Arg	Leu							
			515					520								

<210> 3

<211> 732

<212> PRT

<213> Oryza sativa

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<220>

<221> PEPTIDE

<222> (0)...(0)

<223> Accession No. XP_469850

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<400> 3

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				20					25					30		
	Phe	Ser	Pro	Trp	Asp	Ser	Val	Pro	Leu	Pro	Ala	Thr	Ala	Ser	Pro	Asp
			35					40					45			
	Asp	Gly	Phe	Leu	Leu	Pro	Arg	Phe	Pro	Asp	Val	Cys	Val	Gln	Arg	Ser
15		50					55					60				
	Gln	Phe	Thr	Asn	His	Leu	Ala	Pro	Ala	Asn	Gly	Thr	Gly	Gly	Gly	Gly
	65					70					75					80
	Ser	Arg	Thr	Gly	Val	Lys	Glu	Glu	Ala	Ser	Glu	Val	Leu	Ser	Trp	Pro
					85					90					95	
20	Pro	Thr	Ser	Lys	Gln	Ser	Val	Arg	Arg	Leu	Glu	Val	Ala	Glu	His	Trp
				100					105					110		
	Tyr	Arg	Leu	Tyr	Lys	Thr	Asp	Asn	Gln	Arg	Leu	Ser	Pro	Asp	Ser	Gln
			115					120					125			
	Gln	Val	Ser	Val	Leu	Ala	Glu	Ser	His	Cys	Asp	Leu	Ala	Ser	Gly	Asn
		130					135					140				
25	Trp	Lys	Glu	Ile	Ser	Ile	His	His	Lys	Lys	Met	Pro	Ser	Ser	Thr	Thr
	145					150					155					160
	Thr	Lys	Thr	Thr	Thr	Pro	Ser	Arg	Asp	Ala	Trp	Ile	Val	Ser	Ala	Arg
					165					170					175	
	Ser	Asp	Pro	Phe	His	Leu	Leu	Leu	Glu	Ala	Gln	Ala	Pro	Leu	Gly	Ile
				180					185					190		
30	Lys	Ala	Asp	Ala	Leu	Ser	Gln	Ile	Ala	Ala	Val	His	Gln	Ser	His	Arg
			195					200					205			
	Asn	Thr	Ser	His	Ile	Arg	Glu	Leu	Ser	Leu	Ala	Met	Asp	Asn	Ala	Tyr
		210					215					220				
35	Ile	Ile	Ala	Ile	Leu	Ser	Val	Ala	Ile	Leu	Phe	Leu	Leu	His	Tyr	Tyr
	225					230					235					240
	Leu	Leu	Gly	Arg	Gly	Asn	Gly	Gly	Ala	Ala	Arg	Leu	Pro	Pro	Gly	Pro
					245					250					255	
	Pro	Ala	Val	Pro	Ile	Leu	Gly	His	Leu	His	Leu	Val	Lys	Lys	Pro	Met
				260					265					270		
40	His	Ala	Thr	Met	Ser	Arg	Leu	Ala	Glu	Arg	Tyr	Gly	Pro	Val	Phe	Ser
			275					280					285			
	Leu	Arg	Leu	Gly	Ser	Arg	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Gly	Cys
		290					295					300				
	Ala	Arg	Glu	Cys	Phe	Thr	Glu	His	Asp	Val	Thr	Phe	Ala	Asn	Arg	Pro
	305					310					315					320
45	Arg	Phe	Glu	Ser	Gln	Leu	Leu	Val	Ser	Phe	Asn	Gly	Ala	Ala	Leu	Ala
					325					330					335	
	Thr	Ala	Ser	Tyr	Gly	Ala	His	Trp	Arg	Asn	Leu	Arg	Arg	Ile	Val	Ala
				340					345					350		
	Val	Gln	Leu	Leu	Ser	Ala	His	Arg	Val	Gly	Leu	Met	Ser	Gly	Leu	Ile
			355					360					365			
50	Ala	Gly	Glu	Val	Arg	Ala	Met	Val	Arg	Arg	Met	Tyr	Arg	Ala	Ala	Ala
		370					375					380				
	Ala	Ser	Pro	Ala	Gly	Ala	Ala	Arg	Ile	Gln	Leu	Lys	Arg	Arg	Leu	Phe
	385					390					395					400
55	Glu	Val	Ser	Leu	Ser	Val	Leu	Met	Glu	Thr	Ile	Ala	His	Thr	Lys	Ala
					405					410					415	

Thr Arg Pro Glu Thr Asp Pro Asp Thr Asp Met Ser Val Glu Ala Gln
 420 425 430
 Glu Phe Lys Gln Val Val Asp Glu Ile Ile Pro His Ile Gly Ala Ala
 435 440 445
 5 Asn Leu Trp Asp Tyr Leu Pro Ala Leu Arg Trp Phe Asp Val Phe Gly
 450 455 460
 Val Arg Arg Lys Ile Leu Ala Ala Val Ser Arg Arg Asp Ala Phe Leu
 465 470 475 480
 Arg Arg Leu Ile Asp Ala Glu Arg Arg Arg Leu Asp Asp Gly Asp Glu
 485 490 495
 10 Gly Glu Lys Lys Ser Met Ile Ala Val Leu Leu Thr Leu Gln Lys Thr
 500 505 510
 Glu Pro Glu Val Tyr Thr Asp Asn Met Ile Thr Ala Leu Thr Ala Asn
 515 520 525
 15 Leu Phe Gly Ala Gly Thr Glu Thr Thr Ser Thr Thr Ser Glu Trp Ala
 530 535 540
 Met Ser Leu Leu Leu Asn His Pro Asp Thr Leu Lys Lys Ala Gln Ala
 545 550 555 560
 Glu Ile Asp Ala Ser Val Gly Asn Ser Arg Leu Ile Thr Ala Asp Asp
 565 570 575
 20 Val Thr Arg Leu Gly Tyr Leu Gln Cys Ile Val Arg Glu Thr Leu Arg
 580 585 590
 Leu Tyr Pro Ala Ala Pro Met Leu Leu Pro His Glu Ser Ser Ala Asp
 595 600 605
 Cys Lys Val Gly Gly Tyr Asn Ile Pro Arg Gly Ser Met Leu Leu Ile
 610 615 620
 25 Asn Ala Tyr Ala Ile His Arg Asp Pro Ala Val Trp Glu Glu Pro Glu
 625 630 635 640
 Lys Phe Met Pro Glu Arg Phe Glu Asp Gly Gly Cys Asp Gly Asn Leu
 645 650 655
 30 Leu Met Pro Phe Gly Met Gly Arg Arg Arg Cys Pro Gly Glu Thr Leu
 660 665 670
 Ala Leu Arg Thr Val Gly Leu Val Leu Gly Thr Leu Ile Gln Cys Phe
 675 680 685
 Asp Trp Glu Arg Val Asp Gly Val Glu Val Asp Met Thr Glu Gly Gly
 690 695 700
 35 Gly Leu Thr Ile Pro Lys Val Val Pro Leu Glu Ala Met Cys Arg Pro
 705 710 715 720
 Arg Asp Ala Met Gly Gly Val Leu Arg Glu Leu Val
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<210> 4

<211> 513

<212> PRT

<213> Oryza sativa

<220>

<221> PEPTIDE

<222> (0)...(0)

<223> Accession No.: ABC69856

<400> 4

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 20 25 30
 55 Leu Pro Pro Gly Pro Pro Ala Val Pro Ile Leu Gly His Leu His Leu
 35 40 45
 Val Lys Lys Pro Met His Ala Thr Met Ser Arg Leu Ala Glu Arg Tyr
 50 55 60

	Gly	Pro	Val	Phe	Ser	Leu	Arg	Leu	Gly	Ser	Arg	Arg	Ala	Val	Val	Val
	65					70					75					80
	Ser	Ser	Pro	Gly	Cys	Ala	Arg	Glu	Cys	Phe	Thr	Glu	His	Asp	Val	Thr
5					85					90					95	
	Phe	Ala	Asn	Arg	Pro	Arg	Phe	Glu	Ser	Gln	Leu	Leu	Val	Ser	Phe	Asn
				100					105					110		
	Gly	Ala	Ala	Leu	Ala	Thr	Ala	Ser	Tyr	Gly	Ala	His	Trp	Arg	Asn	Leu
			115					120					125			
10	Arg	Arg	Ile	Val	Ala	Val	Gln	Leu	Leu	Ser	Ala	His	Arg	Val	Gly	Leu
		130					135						140			
	Met	Ser	Gly	Leu	Ile	Ala	Gly	Glu	Val	Arg	Ala	Met	Val	Arg	Arg	Met
	145					150					155					160
	Tyr	Arg	Ala	Ala	Ala	Ala	Ser	Pro	Ala	Gly	Ala	Ala	Arg	Ile	Gln	Leu
					165					170					175	
15	Lys	Arg	Arg	Leu	Phe	Glu	Val	Ser	Leu	Ser	Val	Leu	Met	Glu	Thr	Ile
				180					185					190		
	Ala	His	Thr	Lys	Ala	Thr	Arg	Pro	Glu	Thr	Asp	Pro	Asp	Thr	Asp	Met
			195					200					205			
	Ser	Val	Glu	Ala	Gln	Glu	Phe	Lys	Gln	Val	Val	Asp	Glu	Ile	Ile	Pro
		210					215					220				
20	His	Ile	Gly	Ala	Ala	Asn	Leu	Trp	Asp	Tyr	Leu	Pro	Ala	Leu	Arg	Trp
	225					230					235					240
	Phe	Asp	Val	Phe	Gly	Val	Arg	Arg	Lys	Ile	Leu	Ala	Ala	Val	Ser	Arg
					245					250					255	
25	Arg	Asp	Ala	Phe	Leu	Arg	Arg	Leu	Ile	Asp	Ala	Glu	Arg	Arg	Arg	Leu
				260					265					270		
	Asp	Asp	Gly	Asp	Glu	Gly	Glu	Lys	Lys	Ser	Met	Ile	Ala	Val	Leu	Leu
			275					280					285			
	Thr	Leu	Gln	Lys	Thr	Glu	Pro	Glu	Val	Tyr	Thr	Asp	Asn	Met	Ile	Thr
		290					295					300				
30	Ala	Leu	Thr	Ala	Asn	Leu	Phe	Gly	Ala	Gly	Thr	Glu	Thr	Thr	Ser	Thr
	305					310					315					320
	Thr	Ser	Glu	Trp	Ala	Met	Ser	Leu	Leu	Leu	Asn	His	Pro	Asp	Thr	Leu
					325					330					335	
	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala	Ser	Val	Gly	Asn	Ser	Arg	Leu
				340					345					350		
35	Ile	Thr	Ala	Asp	Asp	Val	Thr	Arg	Leu	Gly	Tyr	Leu	Gln	Cys	Ile	Val
			355					360					365			
	Arg	Glu	Thr	Leu	Arg	Leu	Tyr	Pro	Ala	Ala	Pro	Met	Leu	Leu	Pro	His
		370					375					380				
	Glu	Ser	Ser	Ala	Asp	Cys	Lys	Val	Gly	Gly	Tyr	Asn	Ile	Pro	Arg	Gly
	385					390					395					400
40	Ser	Met	Leu	Leu	Ile	Asn	Ala	Tyr	Ala	Ile	His	Arg	Asp	Pro	Ala	Val
					405					410					415	
	Trp	Glu	Glu	Pro	Glu	Lys	Phe	Met	Pro	Glu	Arg	Phe	Glu	Asp	Gly	Gly
				420					425					430		
	Cys	Asp	Gly	Asn	Leu	Leu	Met	Pro	Phe	Gly	Met	Gly	Arg	Arg	Arg	Cys
			435					440					445			
45	Pro	Gly	Glu	Thr	Leu	Ala	Leu	Arg	Thr	Val	Gly	Leu	Val	Leu	Gly	Thr
		450					455					460				
	Leu	Ile	Gln	Cys	Phe	Asp	Trp	Glu	Arg	Val	Asp	Gly	Val	Glu	Val	Asp
	465					470					475					480
50	Met	Thr	Glu	Gly	Gly	Gly	Leu	Thr	Ile	Pro	Lys	Val	Val	Pro	Leu	Glu
					485					490					495	
	Ala	Met	Cys	Arg	Pro	Arg	Asp	Ala	Met	Gly	Gly	Val	Leu	Arg	Glu	Leu
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	Val															

<210> 5

<211> 517

<212> PRT

<213> Lolium rigidum

<220>

<221> PEPTIDE

<222> (0)...(0)

<223> Accession No.: AAK38080

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<400> 5

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10	Val	His	Tyr	Val	Leu	Gly	Lys	Val	Ser	Asp	Gly	Arg	Arg	Gly	Lys	Lys
				20					25					30		
	Gly	Ala	Val	Gln	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Val	Pro	Phe	Leu	Gly
			35					40					45			
15	His	Leu	His	Leu	Val	Asp	Lys	Pro	Ile	His	Ala	Thr	Met	Cys	Arg	Leu
	50						55					60				
	Ala	Ala	Arg	Leu	Gly	Pro	Val	Phe	Ser	Leu	Arg	Leu	Gly	Ser	Arg	Arg
	65					70					75					80
	Ala	Val	Val	Val	Ser	Ser	Ser	Glu	Cys	Ala	Arg	Glu	Cys	Phe	Thr	Glu
					85					90					95	
20	His	Asp	Val	Thr	Phe	Ala	Asn	Arg	Pro	Lys	Phe	Pro	Ser	Gln	Leu	Leu
				100					105					110		
	Val	Ser	Phe	Asn	Gly	Thr	Ala	Leu	Val	Thr	Ser	Ser	Tyr	Gly	Pro	His
			115					120					125			
	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Thr	Val	Gln	Leu	Leu	Ser	Ala	His
	130						135					140				
25	Arg	Val	Ala	Cys	Met	Ser	Gly	Val	Ile	Ala	Ala	Glu	Val	Arg	Ala	Met
	145					150					155					160
	Ala	Arg	Arg	Leu	Phe	His	Ala	Thr	Glu	Ala	Ser	Pro	Asp	Gly	Ala	Ala
					165					170					175	
	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	Ser	Val	Leu
				180					185					190		
30	Met	Glu	Thr	Ile	Ala	Gln	Thr	Lys	Ala	Thr	Arg	Ser	Glu	Ala	Asp	Ala
			195					200					205			
	Asp	Thr	Asp	Met	Ser	Val	Glu	Ala	Gln	Glu	Phe	Lys	Glu	Val	Val	Asp
	210						215					220				
35	Lys	Leu	Ile	Pro	His	Leu	Gly	Ala	Ala	Asn	Met	Trp	Asp	Tyr	Leu	Pro
	225					230				235						240
	Val	Met	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys	Ile	Leu	His
				245					250					255		
	Ala	Val	Ser	Arg	Arg	Asp	Ala	Phe	Leu	Arg	Arg	Leu	Ile	Asp	Ala	Glu
				260					265					270		
40	Arg	Arg	Arg	Leu	Ala	Asp	Gly	Gly	Ser	Asp	Gly	Asp	Lys	Lys	Ser	Met
				275				280					285			
	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Thr	Glu	Pro	Lys	Val	Tyr	Thr
	290						295					300				
	Asp	Thr	Met	Ile	Thr	Ala	Leu	Cys	Ala	Asn	Leu	Phe	Gly	Ala	Gly	Thr
	305					310				315						320
45	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu	Leu	Asn
				325						330				335		
	His	Pro	Ala	Ala	Leu	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala	Ser	Val
				340					345					350		
	Gly	Thr	Ser	Arg	Leu	Val	Ser	Val	Asp	Asp	Val	Pro	Ser	Leu	Ala	Tyr
			355					360					365			
50	Leu	Gln	Cys	Ile	Val	Ser	Glu	Thr	Leu	Arg	Leu	Tyr	Pro	Ala	Ala	Pro
	370						375					380				
	Leu	Leu	Leu	Pro	His	Glu	Ser	Ser	Ala	Asp	Cys	Lys	Val	Gly	Gly	Tyr
	385					390					395					400

55

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	Asn	Val	Pro	Ala	Asp	Thr	Met	Leu	Ile	Val	Asn	Ala	Tyr	Ala	Ile	His
					405					410					415	
	Arg	Asp	Pro	Ala	Ala	Trp	Glu	Asp	Pro	Leu	Glu	Phe	Arg	Pro	Glu	Arg
				420				425						430		
5	Phe	Glu	Asp	Gly	Lys	Ala	Glu	Gly	Leu	Phe	Met	Ile	Pro	Phe	Gly	Met
			435					440					445			
	Gly	Arg	Arg	Arg	Cys	Pro	Gly	Glu	Thr	Leu	Ala	Leu	Arg	Thr	Ile	Gly
		450					455					460				
	Met	Val	Leu	Ala	Thr	Leu	Val	Gln	Cys	Phe	Asp	Trp	Glu	Pro	Val	Asp
10	465					470					475					480
	Gly	Val	Lys	Val	Asp	Met	Thr	Glu	Gly	Gly	Gly	Phe	Thr	Ile	Pro	Lys
					485					490					495	
	Ala	Val	Pro	Leu	Glu	Ala	Val	Cys	Arg	Pro	Arg	Ala	Val	Met	Arg	Asp
				500					505					510		
	Val	Leu	Gln	Asn	Leu											
15			515													

<210> 6

<211> 517

<212> PRT

20 <213> Lolium rigidum

<220>

<221> PEPTIDE

<222> (0)...(0)

25 <223> Accession No.: AAK38079

<400> 6

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	Val	His	Tyr	Val	Leu	Gly	Lys	Val	Ser	Asp	Gly	Arg	Arg	Gly	Lys	Lys
				20					25					30		
	Gly	Ala	Val	Gln	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Val	Pro	Phe	Leu	Gly
			35					40					45			
35	His	Leu	His	Leu	Val	Glu	Lys	Pro	Ile	His	Ala	Thr	Met	Cys	Arg	Leu
	50					55					60					
	Ala	Ala	Arg	Leu	Gly	Pro	Val	Phe	Ser	Leu	Arg	Leu	Gly	Ser	Arg	Arg
	65				70					75						80
	Ala	Val	Val	Val	Ser	Ser	Glu	Cys	Ala	Arg	Glu	Cys	Phe	Thr	Glu	
					85				90					95		
40	His	Asp	Val	Thr	Phe	Ala	Asn	Arg	Pro	Lys	Phe	Pro	Ser	Gln	Leu	Leu
				100					105					110		
	Val	Ser	Phe	Asn	Gly	Thr	Ala	Leu	Val	Thr	Ser	Ser	Tyr	Gly	Pro	His
			115					120					125			
	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Thr	Val	Gln	Leu	Leu	Ser	Ala	His
45	130						135					140				
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	145				150					155						160
	Ala	Arg	Arg	Leu	Phe	His	Ala	Ala	Glu	Ala	Ser	Pro	Asp	Gly	Ala	Ala
					165					170					175	
50	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	Ser	Val	Leu
				180					185					190		
	Met	Glu	Thr	Ile	Ala	Gln	Thr	Lys	Ala	Thr	Arg	Ser	Glu	Ala	Asp	Ala
			195					200					205			
	Asp	Thr	Asp	Met	Ser	Leu	Glu	Ala	Gln	Glu	Phe	Lys	Glu	Val	Val	Asp
		210					215					220				
55	Lys	Leu	Ile	Pro	His	Leu	Gly	Ala	Ala	Asn	Met	Trp	Asp	Tyr	Leu	Pro
	225					230					235					240
	Val	Met	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Ser	Lys	Ile	Leu	His
					245					250					255	

Ala Val Ser Arg Arg Asp Ala Phe Leu Arg Arg Leu Ile Asn Ala Glu
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 Arg Arg Arg Leu Ala Asp Gly Gly Ser Asp Gly Asp Lys Lys Ser Met
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 5 Ile Ala Val Leu Leu Thr Leu Gln Lys Thr Glu Pro Lys Val Tyr Thr
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 Asp Thr Met Ile Thr Ala Leu Cys Ala Asn Leu Phe Gly Ala Gly Thr
 305 310 315 320
 10 Glu Thr Thr Ser Thr Thr Thr Glu Trp Ala Met Ser Leu Leu Leu Asn
 325 330 335
 His Pro Ala Ala Leu Lys Lys Ala Gln Ala Glu Ile Asp Ala Ser Val
 340 345 350
 Gly Thr Ser Arg Leu Val Ser Val Asp Asp Val Pro Ser Leu Ala Tyr
 355 360 365
 15 Leu Gln Cys Ile Val Ser Glu Thr Leu Arg Leu Tyr Pro Ala Ala Pro
 370 375 380
 Leu Leu Leu Pro His Glu Ser Ser Ala Asp Cys Lys Val Gly Gly Tyr
 385 390 395 400
 Asn Val Pro Ala Asp Thr Met Leu Ile Val Asn Ala Tyr Ala Ile His
 405 410 415
 20 Arg Asp Pro Ala Ala Trp Glu Asp Pro Leu Glu Phe Lys Pro Glu Arg
 420 425 430
 Phe Glu Asp Gly Lys Ala Glu Gly Leu Phe Met Ile Pro Phe Gly Met
 435 440 445
 Gly Arg Arg Arg Cys Pro Gly Glu Thr Leu Ala Leu Arg Thr Ile Gly
 450 455 460
 25 Met Val Leu Ala Thr Leu Val Gln Cys Phe Asp Trp Glu Pro Val Asp
 465 470 475 480
 Gly Val Lys Val Asp Met Thr Glu Gly Gly Phe Thr Ile Pro Lys
 485 490 495
 30 Ala Val Pro Leu Glu Ala Val Cys Arg Pro Arg Val Val Met Arg Asp
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 Val Leu Gln Asn Leu
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 <223> AAK38081

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 20 25 30
 50 Gly Ala Val Gln Leu Pro Pro Ser Pro Pro Ala Val Pro Phe Leu Gly
 35 40 45
 His Leu His Leu Val Asp Lys Pro Ile His Ala Thr Met Cys Arg Leu
 50 55 60
 Ala Ala Arg Leu Gly Pro Val Phe Ser Leu Arg Leu Gly Ser Arg Arg
 65 70 75 80
 55 Ala Val Val Val Ser Ser Ser Glu Cys Ala Arg Glu Cys Phe Thr Glu
 85 90 95
 His Asp Val Thr Phe Ala Asn Arg Pro Lys Phe Pro Ser Gln Leu Leu
 100 105 110

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	Val	Ser	Phe	Asn	Gly	Thr	Ala	Leu	Val	Thr	Ser	Ser	Tyr	Gly	Pro	His
			115					120					125			
	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Thr	Val	Gln	Leu	Leu	Ser	Ala	His
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5	Arg	Val	Ala	Cys	Met	Ser	Gly	Val	Ile	Ala	Ala	Glu	Val	Arg	Ala	Met
	145					150					155					160
	Ala	Arg	Arg	Leu	Phe	His	Ala	Ala	Glu	Ala	Ser	Pro	Asp	Gly	Ala	Ala
				165						170					175	
	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	Ser	Val	Leu
10				180					185					190		
	Met	Glu	Thr	Ile	Ala	Gln	Thr	Lys	Ala	Thr	Arg	Ser	Glu	Ala	Asp	Ala
			195					200					205			
	Asp	Thr	Asp	Met	Ser	Val	Glu	Ala	Gln	Glu	Phe	Lys	Glu	Val	Val	Asp
		210					215					220				
15	Lys	Leu	Ile	Pro	His	Leu	Gly	Ala	Ala	Asn	Met	Trp	Asp	Tyr	Leu	Pro
	225					230					235					240
	Val	Met	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys	Ile	Leu	His
				245					250					255		
	Ala	Val	Ser	Arg	Arg	Asp	Ala	Phe	Leu	Arg	Arg	Leu	Ile	Asp	Ala	Glu
				260					265					270		
20	Arg	Arg	Arg	Leu	Ala	Asp	Gly	Gly	Ser	Asp	Gly	Asp	Lys	Lys	Ser	Met
			275					280					285			
	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Thr	Glu	Pro	Lys	Val	Tyr	Thr
		290					295					300				
	Asp	Thr	Met	Ile	Thr	Ala	Leu	Cys	Ala	Asn	Leu	Phe	Gly	Ala	Gly	Thr
	305					310					315					320
25	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu	Leu	Asn
				325					330					335		
	His	Pro	Ala	Ala	Leu	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala	Ser	Val
				340					345					350		
	Gly	Thr	Ser	Arg	Leu	Val	Ser	Val	Asp	Asp	Val	Pro	Ser	Leu	Ala	Tyr
			355					360					365			
30	Leu	Gln	Cys	Ile	Val	Asn	Glu	Thr	Leu	Arg	Leu	Tyr	Pro	Ala	Ala	Pro
		370					375					380				
	Leu	Leu	Leu	Pro	His	Glu	Ser	Ser	Ala	Asp	Cys	Lys	Val	Gly	Gly	Tyr
	385					390					395					400
35	Asn	Val	Pro	Ala	Asp	Thr	Met	Leu	Ile	Val	Asn	Ala	Tyr	Ala	Ile	His
				405						410					415	
	Arg	Asp	Pro	Ala	Ala	Trp	Glu	His	Pro	Leu	Val	Phe	Arg	Pro	Glu	Arg
				420					425					430		
	Phe	Glu	Asp	Gly	Lys	Ala	Glu	Gly	Leu	Phe	Met	Ile	Pro	Phe	Gly	Met
			435					440					445			
40	Gly	Arg	Arg	Arg	Cys	Pro	Gly	Glu	Thr	Leu	Ala	Leu	Arg	Thr	Ile	Gly
		450					455					460				
	Met	Val	Leu	Ala	Thr	Leu	Val	Gln	Cys	Phe	Asp	Trp	Glu	Pro	Val	Asp
	465					470					475					480
	Gly	Val	Asn	Val	Asp	Met	Thr	Glu	Gly	Gly	Gly	Phe	Thr	Ile	Pro	Lys
				485						490					495	
45	Ala	Val	Pro	Leu	Glu	Ala	Val	Cys	Arg	Pro	Arg	Ala	Val	Met	Arg	Asp
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55 <220>
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 <222> (0)...(0)
 <223> Accession No.: BAD27508

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				20					25					30		
	Gly	Ala	Val	Gln	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Ile	Pro	Phe	Ile	Gly
			35					40					45			
10	His	Leu	His	Leu	Val	Glu	Lys	Pro	Ile	His	Ala	Thr	Met	Cys	Arg	Leu
	50					55					60					
	Ala	Ala	Arg	Leu	Gly	Pro	Val	Phe	Ser	Leu	Arg	Leu	Gly	Ser	Arg	Arg
	65				70					75					80	
	Ala	Val	Val	Val	Pro	Ser	Ser	Glu	Cys	Ala	Arg	Glu	Cys	Phe	Thr	Glu
				85					90					95		
15	His	Asp	Val	Thr	Phe	Ala	Asn	Arg	Pro	Lys	Phe	Pro	Ser	Gln	Leu	Leu
				100					105					110		
	Ala	Ser	Phe	Asn	Gly	Thr	Ala	Leu	Val	Thr	Ser	Ser	Tyr	Gly	Pro	His
			115					120					125			
	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Thr	Val	Gln	Leu	Leu	Ser	Ala	His
	130						135					140				
20	Arg	Val	Ala	Cys	Met	Ser	Gly	Val	Ile	Ala	Ala	Glu	Val	Arg	Ala	Met
	145				150					155						160
	Ala	Arg	Arg	Leu	Phe	His	Ala	Ala	Glu	Ala	Ser	Pro	Asp	Gly	Ala	Ala
				165					170					175		
	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	Ser	Val	Leu
				180					185					190		
25	Met	Glu	Thr	Ile	Ala	Gln	Thr	Lys	Ala	Thr	Arg	Ser	Glu	Ala	Asp	Ala
			195					200					205			
	Asp	Thr	Asp	Met	Ser	Val	Glu	Ala	Gln	Glu	Phe	Lys	Glu	Val	Val	Asp
		210				215						220				
30	Lys	Leu	Ile	Pro	His	Leu	Gly	Ala	Ala	Asn	Met	Trp	Asp	Tyr	Leu	Pro
	225				230					235					240	
	Val	Met	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys	Ile	Leu	His
				245					250					255		
	Ala	Val	Ser	Arg	Arg	Asp	Ala	Phe	Leu	Arg	Arg	Leu	Ile	Asp	Ala	Glu
				260				265						270		
35	Arg	Arg	Arg	Leu	Ala	Asp	Gly	Gly	Ser	Asp	Gly	Asp	Lys	Lys	Ser	Met
			275					280					285			
	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Thr	Glu	Pro	Lys	Val	Tyr	Thr
		290				295						300				
	Asp	Thr	Met	Ile	Thr	Ala	Leu	Cys	Ala	Asn	Leu	Phe	Gly	Ala	Gly	Thr
	305				310					315					320	
40	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu	Leu	Asn
				325					330					335		
	His	Pro	Ala	Ala	Leu	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala	Ser	Val
				340					345					350		
	Gly	Thr	Ser	Arg	Leu	Val	Ser	Val	Asp	Asp	Val	Pro	Ser	Leu	Ala	Tyr
			355					360					365			
45	Leu	Gln	Cys	Ile	Val	Asn	Glu	Thr	Leu	Arg	Leu	Tyr	Pro	Ala	Ala	Pro
		370				375						380				
	Leu	Leu	Leu	Pro	His	Glu	Ser	Ser	Ala	Asp	Cys	Lys	Val	Gly	Gly	Tyr
	385				390					395					400	
50	Asn	Val	Pro	Ala	Asp	Thr	Met	Leu	Ile	Val	Asn	Ala	Tyr	Ala	Ile	His
				405						410					415	
	Arg	Asp	Pro	Ala	Ala	Trp	Glu	His	Pro	Leu	Glu	Phe	Arg	Pro	Glu	Arg
				420				425						430		
	Phe	Glu	Asp	Gly	Lys	Ala	Glu	Gly	Leu	Phe	Met	Ile	Pro	Phe	Gly	Val
			435					440					445			

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	Gly	Arg	Arg	Arg	Cys	Pro	Gly	Glu	Thr	Leu	Ala	Leu	Arg	Thr	Ile	Ser
		450					455					460				
	Met	Val	Leu	Ala	Thr	Leu	Val	Gln	Cys	Phe	Asp	Trp	Glu	Pro	Val	Asp
	465					470					475					480
5	Gly	Val	Lys	Val	Asp	Met	Thr	Glu	Gly	Gly	Gly	Phe	Thr	Ile	Pro	Lys
					485					490					495	
	Ala	Val	Pro	Leu	Glu	Ala	Val	Cys	Arg	Pro	Arg	Ala	Val	Met	Arg	Asp
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<212> PRT

15 <213> Lolium rigidum

<220>

<221> PEPTIDE

<222> (0)...(0)

20 <223> Accession No.: BAD27507

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	Val	His	Tyr	Val	Leu	Gly	Lys	Val	Ser	His	Gly	Arg	Arg	Gly	Lys	Lys
				20					25					30		
	Gly	Ala	Val	Gln	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Ile	Pro	Phe	Ile	Gly
			35					40					45			
30	His	Leu	His	Leu	Val	Glu	Lys	Pro	Ile	His	Ala	Thr	Met	Cys	Arg	Leu
	50					55						60				
	Ala	Ala	Arg	Leu	Gly	Pro	Val	Phe	Ser	Leu	Arg	Leu	Gly	Ser	Arg	Arg
	65				70						75					80
	Ala	Val	Val	Val	Ser	Ser	Ser	Glu	Cys	Ala	Arg	Glu	Cys	Phe	Thr	Glu
					85					90					95	
35	His	Asp	Val	Thr	Phe	Ala	Asn	Arg	Pro	Ser	Ser	Arg	Arg	Lys	Leu	Leu
				100					105					110		
	Ala	Ser	Phe	Asn	Gly	Thr	Ala	Leu	Val	Thr	Ser	Ser	Tyr	Gly	Pro	His
			115					120					125			
	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Thr	Val	Gln	Leu	Leu	Ser	Ala	His
40							135					140				
	Arg	Val	Ala	Cys	Met	Ser	Gly	Val	Ile	Ala	Ala	Glu	Val	Arg	Ala	Met
	145				150						155					160
	Ala	Arg	Arg	Leu	Phe	His	Ala	Ala	Glu	Ala	Ser	Pro	Asp	Gly	Ala	Thr
					165					170					175	
45	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	Ser	Val	Leu
				180					185					190		
	Met	Glu	Thr	Ile	Ala	Gln	Thr	Lys	Ala	Thr	Arg	Ser	Glu	Ala	Asp	Ala
			195					200					205			
	Asp	Thr	Asp	Met	Ser	Val	Glu	Ala	Gln	Glu	Phe	Lys	Glu	Val	Val	Asp
	210					215						220				
50	Lys	Leu	Ile	Pro	His	Leu	Gly	Ala	Ala	Asn	Met	Trp	Asp	Tyr	Leu	Pro
	225					230					235					240
	Val	Met	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys	Ile	Leu	His
					245					250					255	
	Ala	Val	Ser	Arg	Arg	Asp	Ala	Phe	Leu	Arg	Arg	Leu	Ile	Asp	Ala	Glu
				260					265					270		
55	Arg	Arg	Arg	Leu	Ala	Asp	Gly	Gly	Ser	Asp	Gly	Asp	Lys	Lys	Ser	Met
			275					280					285			
	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Thr	Glu	Pro	Lys	Val	Tyr	Thr
		290					295					300				

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	Asp	Thr	Met	Ile	Thr	Ala	Leu	Cys	Ala	Asn	Leu	Phe	Gly	Ala	Gly	Thr
	305					310					315					320
	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu	Leu	Asn
					325					330						335
5	His	Pro	Ala	Ala	Leu	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala	Ser	Val
				340					345					350		
	Gly	Thr	Ser	Arg	Leu	Val	Ser	Val	Asp	Asp	Val	Leu	Ser	Leu	Ala	Tyr
			355					360					365			
	Leu	Gln	Cys	Ile	Val	Ser	Glu	Thr	Leu	Arg	Leu	Tyr	Pro	Ala	Ala	Pro
10		370					375					380				
	Leu	Leu	Leu	Pro	His	Glu	Ser	Ser	Ala	Asp	Cys	Lys	Val	Gly	Gly	Tyr
	385					390					395					400
	Asn	Val	Pro	Ala	Asp	Thr	Met	Leu	Ile	Val	Asn	Ala	Tyr	Ala	Ile	His
					405					410						415
	Arg	Asp	Pro	Ala	Ala	Trp	Glu	His	Pro	Leu	Glu	Phe	Arg	Pro	Glu	Arg
15				420					425					430		
	Phe	Glu	Asp	Gly	Lys	Ala	Glu	Gly	Leu	Phe	Met	Ile	Pro	Phe	Gly	Met
			435					440					445			
	Gly	Arg	Arg	Arg	Cys	Pro	Gly	Glu	Thr	Leu	Ala	Leu	Arg	Thr	Ile	Gly
		450					455					460				
20	Met	Val	Leu	Ala	Thr	Leu	Val	Gln	Cys	Phe	Asp	Trp	Glu	Pro	Val	Asp
	465					470					475					480
	Gly	Val	Lys	Val	Asp	Met	Thr	Glu	Gly	Gly	Gly	Phe	Thr	Ile	Pro	Lys
					485					490					495	
	Ala	Val	Pro	Leu	Glu	Ala	Val	Cys	Arg	Pro	Arg	Thr	Val	Met	Arg	Asp
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25	Val	Leu	Gln	Asn	Leu											
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30 <212> PRT

<213> Lolium rigidum

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<221> PEPTIDE

35 <222> (0)...(0)

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	Val	His	Tyr	Val	Leu	Gly	Lys	Val	Ser	His	Gly	Arg	Arg	Gly	Lys	Lys
				20					25					30		
45	Gly	Ala	Val	Gln	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Ile	Pro	Phe	Ile	Gly
			35					40					45			
	His	Leu	His	Leu	Val	Glu	Lys	Pro	Ile	His	Ala	Thr	Met	Cys	Arg	Leu
		50					55					60				
	Ala	Ala	Arg	Leu	Gly	Pro	Val	Phe	Ser	Leu	Arg	Leu	Gly	Ser	Arg	Arg
	65					70					75					80
50	Ala	Val	Val	Val	Ser	Ser	Glu	Cys	Ala	Arg	Glu	Cys	Phe	Thr	Glu	
					85				90					95		
	His	Asp	Val	Thr	Phe	Ala	Asn	Arg	Pro	Lys	Phe	Pro	Ser	Gln	Leu	Leu
				100					105					110		
	Ala	Ser	Phe	Asn	Gly	Thr	Ala	Leu	Val	Thr	Pro	Ser	Tyr	Gly	Pro	His
			115					120					125			
55	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Thr	Val	Gln	Leu	Leu	Ser	Ala	His
		130					135					140				
	Arg	Val	Ala	Cys	Met	Ser	Gly	Val	Ile	Ala	Ala	Glu	Val	Arg	Ala	Met
	145					150					155					160

Ala Arg Arg Leu Phe His Ala Ala Glu Ala Ser Pro Gly Gly Ala Ala
 165 170 175
 Arg Val Gln Leu Lys Arg Gly Pro Phe Glu Leu Ser Leu Ser Val Leu
 180 185 190
 5 Met Glu Thr Ile Ala Gln Thr Lys Ala Thr Arg Ser Glu Ala Asp Ala
 195 200 205
 Asp Thr Asp Met Ser Val Glu Ala Gln Glu Phe Lys Glu Val Val Asp
 210 215 220
 10 Lys Pro Ile Pro His Leu Gly Ala Ala Asn Met Trp Asp Tyr Leu Pro
 225 230 235 240
 Val Met Arg Trp Phe Asp Val Phe Gly Val Arg Asn Lys Ile Leu His
 245 250 255
 Ala Val Ser Arg Arg Asp Ala Phe Leu Arg Arg Leu Ile Asp Ala Glu
 260 265 270
 15 Arg Arg Arg Leu Ala Asp Gly Gly Ser Asp Gly Asp Lys Lys Ser Met
 275 280 285
 Ile Ala Val Leu Leu Thr Leu Gln Lys Thr Glu Pro Lys Val Tyr Thr
 290 295 300
 Asp Thr Met Ile Thr Ala Leu Cys Ala Asn Leu Phe Gly Ala Gly Thr
 305 310 315 320
 20 Glu Thr Thr Ser Thr Thr Thr Glu Arg Ala Met Ser Leu Leu Leu Asn
 325 330 335
 His Pro Ala Ala Leu Lys Lys Ala Gln Ala Glu Ile Asp Ala Ser Val
 340 345 350
 Gly Thr Ser Arg Leu Val Ser Val Asp Asp Met Pro Ser Leu Ala Tyr
 355 360 365
 25 Leu Gln Cys Ile Val Asn Glu Thr Leu Arg Leu Tyr Pro Ala Ala Pro
 370 375 380
 Leu Leu Leu Pro His Glu Ser Ser Ala Asp Cys Lys Val Gly Gly Tyr
 385 390 395 400
 30 Asn Val Pro Ala Asp Thr Met Leu Ile Val Asn Ala Tyr Ala Ile His
 405 410 415
 Arg Asp Pro Ala Ala Trp Glu His Pro Leu Glu Phe Arg Pro Glu Arg
 420 425 430
 Phe Glu Asp Gly Lys Ala Glu Gly Leu Phe Met Ile Pro Phe Gly Met
 435 440 445
 35 Gly Arg Arg Arg Cys Pro Gly Glu Thr Leu Ala Leu Arg Thr Ile Gly
 450 455 460
 Met Val Leu Ala Thr Leu Val Gln Cys Phe Asp Trp Glu Pro Val Asp
 465 470 475 480
 Gly Val Lys Val Asp Met Thr Glu Gly Gly Gly Phe Thr Ile Pro Lys
 485 490 495
 40 Ala Val Pro Leu Glu Ala Val Cys Arg Pro Arg Ala Val Met Arg Asp
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 Val Leu Gln Asn Leu
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45 <210> 11
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 <213> Oryza sativa

50 <220>
 <221> PEPTIDE
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 <223> Accession No.: XP_469849

55 <400> 11

Met Asp Lys Ala Tyr Ile Ala Val Phe Ser Ile Val Ile Leu Phe Leu
 1 5 10 15

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	Leu	Val	Asp	Tyr	Leu	Arg	Arg	Leu	Arg	Gly	Gly	Gly	Thr	Ser	Asn	Gly
				20					25					30		
	Lys	Asn	Lys	Gly	Met	Arg	Leu	Pro	Pro	Gly	Leu	Pro	Ala	Val	Pro	Ile
		35						40					45			
5	Ile	Gly	His	Leu	His	Leu	Val	Lys	Lys	Pro	Met	His	Ala	Thr	Leu	Ser
		50					55					60				
	Arg	Leu	Ala	Ala	Arg	His	Gly	Pro	Val	Phe	Ser	Leu	Arg	Leu	Gly	Ser
	65					70					75					80
	Arg	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Gly	Cys	Ala	Arg	Glu	Cys	Phe
10					85					90					95	
	Thr	Glu	His	Asp	Val	Ala	Phe	Ala	Asn	Arg	Pro	Arg	Phe	Glu	Ser	Gln
				100					105					110		
	Leu	Leu	Met	Ser	Phe	Asp	Gly	Thr	Ala	Leu	Ala	Met	Ala	Ser	Tyr	Gly
			115					120					125			
15	Pro	His	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Ala	Val	Gln	Leu	Leu	Ser
		130					135					140				
	Ala	Arg	Arg	Val	Gly	Leu	Met	Ser	Gly	Leu	Ile	Ala	Gly	Glu	Val	Arg
						150					155					160
	Ala	Met	Val	Arg	Ser	Leu	Cys	Arg	Arg	Pro	Ala	Ala	Ala	Ala	Pro	Val
					165					170					175	
20	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	Ser	Val	Leu	Met	Glu
				180					185					190		
	Thr	Ile	Ala	Gln	Ser	Lys	Ala	Thr	Arg	Pro	Glu	Thr	Thr	Asp	Thr	Asp
			195					200					205			
	Thr	Asp	Met	Ser	Met	Glu	Ala	Gln	Glu	Tyr	Lys	Gln	Val	Val	Glu	Glu
		210					215					220				
25	Ile	Leu	Glu	Arg	Ile	Gly	Thr	Gly	Asn	Leu	Cys	Asp	Tyr	Leu	Pro	Ala
		225				230					235					240
	Leu	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Arg	Ile	Leu	Ala	Ala
				245					250						255	
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				260					265					270		
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			275					280					285			
	Thr	Leu	Gln	Lys	Thr	Gln	Pro	Glu	Val	Tyr	Thr	Asp	Asn	Met	Ile	Thr
		290					295					300				
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40	Ile	Thr	Ala	Asp	Asp	Val	Pro	Arg	Ile	Thr	Tyr	Leu	Gln	Cys	Ile	Val
			355					360					365			
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		385				390					395					400
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					405					410					415	
	Trp	Glu	Glu	Pro	Glu	Arg	Phe	Val	Pro	Glu	Arg	Phe	Glu	Gly	Gly	Gly
				420					425					430		
	Cys	Asp	Gly	Asn	Leu	Ser	Met	Pro	Phe	Gly	Met	Gly	Arg	Arg	Arg	Cys
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			450				455					460				
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						470					475					480
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5 <210> 12
<211> 527
<212> PRT
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<222> (0)...(0)
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	Leu	Gly	Ser	Arg	Asn	Ala	Val	Val	Val	Ser	Ser	Pro	Glu	Cys	Ala	Arg
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35	Glu	Ile	Arg	Pro	Met	Val	Gln	Arg	Met	Tyr	Arg	Ala	Ala	Ala	Ala	Ala
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	225					230					235					240
45	Leu	Trp	Asp	Tyr	Leu	Pro	Ala	Leu	Arg	Val	Phe	Asp	Val	Leu	Gly	Val
					245					250					255	
	Lys	Arg	Lys	Ile	Ala	Thr	Leu	Ala	Asn	Arg	Arg	Asp	Ala	Phe	Val	Arg
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	Gln	Lys	Thr	Glu	Pro	Glu	Val	Tyr	Lys	Asp	Ile	Val	Ile	Val	Asn	Leu
		305				310					315					320
	Cys	Ala	Ala	Leu	Phe	Ala	Ala	Gly	Thr	Glu	Thr	Thr	Ala	Met	Thr	Ile
					325					330					335	
55	Glu	Trp	Ala	Met	Ser	Leu	Leu	Leu	Asn	His	Pro	Lys	Ile	Leu	Lys	Lys
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	Leu	Leu	Val	Asn	Val	Ile	Ala	Ile	Gln	Arg	Asp	Pro	Met	Val	Trp	Lys
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			435					440					445			
10	Gly	Leu	Phe	Met	Ile	Pro	Phe	Gly	Met	Gly	Arg	Arg	Lys	Cys	Pro	Gly
	450						455					460				
	Glu	Thr	Met	Ala	Leu	Gln	Thr	Ile	Gly	Leu	Val	Leu	Gly	Ala	Leu	Ile
	465					470					475					480
	Gln	Cys	Phe	Asp	Trp	Asp	Arg	Val	Asp	Gly	Ala	Glu	Val	Asp	Met	Thr
15				485						490					495	
	Gln	Gly	Ser	Gly	Leu	Thr	Asn	Pro	Arg	Ala	Val	Pro	Leu	Glu	Ala	Met
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<211> 518

<212> PRT

<213> Oryza sativa

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<220>

<221> PEPTIDE

<222> (0)...(0)

<223> Accession No.: XP_469852

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<400> 13

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55	Gln	Ser	Lys	Thr	Thr	Arg	Arg	Glu	Ala	Pro	Asp	Ala	Asp	Thr	Asp	Met
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	Glu	Thr	Thr	Ser	Thr	Thr	Ile	Glu	Trp	Ala	Met	Ser	Leu	Leu	Leu	Asn
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20	Leu	Leu	Leu	Pro	His	Glu	Ser	Ser	Ala	Asp	Cys	Lys	Val	Gly	Gly	Tyr
	385					390					395					400
	His	Ile	Pro	Ser	Gly	Ala	Met	Leu	Leu	Val	Asn	Val	Ala	Ala	Ile	Gln
					405					410					415	
	Arg	Asp	Pro	Val	Ile	Trp	Lys	Glu	Pro	Ser	Glu	Phe	Lys	Pro	Glu	Arg
				420					425					430		
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			435					440					445			
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		450					455				460					
	Leu	Val	Leu	Gly	Thr	Met	Ile	Gln	Cys	Phe	Asp	Trp	Gly	Arg	Val	Asp
	465					470					475					480
30	Asp	Ala	Met	Val	Asp	Met	Thr	Gln	Ser	Asn	Gly	Leu	Thr	Ser	Leu	Lys
					485					490					495	
	Val	Ile	Pro	Leu	Glu	Ala	Met	Cys	Lys	Pro	Arg	Glu	Ala	Met	Cys	Asp
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<223> Xaa = Any Amino Acid

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<223> Motif common to all Cytochrome P450 sequences

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<223> Xaa = Ala or Gly

<221> VARIANT

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$\langle 222 \rangle$ (6)...(6)

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<213> Zea mays

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<223> Maize B73 genomic sequence for Nsf1 gene

<221> intron

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<400> 16

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	ccgccgagcc	ctccggcgat	cccggttcctg	ggccacctcc	acctcgtcaa	ggccccgttc	180
5	cacggggcgc	tggcccgcct	cgcgggcgcg	cacggcccgg	tgttctccat	gcgcctgggg	240
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	gacgtgaact	tgcggaaccg	gccgctgttc	ccgtcgatgc	ggctggcgtc	cttcgacggc	360
	gccatgctct	ccgtgtccag	ctacggcccc	tactggcgca	acctgcgccc	cgtcgcgcgc	420
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25	acgacgtgcc	ccacctcacc	tacctgcagt	gcacgtcgca	cgagacgctg	cgcctgcacc	1440
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 <223> Q66 maize line ORF for Nsf1

<221> CDS
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<400> 17

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5	ctc	cac	tac	ctc	ctg	ggc	cgg	cgg	gcc	ggc	ggc	gag	ggc	aag	gcc	aag	96
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10	gcc	aag	ggc	tgc	cgg	cgg	cgg	ctc	ccg	ccg	agc	cct	ccg	gcg	atc	ccg	144
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	Ala	Arg	Leu	Ala	Ala	Arg	His	Gly	Pro	Val	Phe	Ser	Met	Arg	Leu	Gly	
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25	acc	cgg	cgc	gcc	gtg	gtc	gtg	tgc	tgc	ccg	gac	tgc	gcc	agg	gag	tgc	288
	Thr	Arg	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Asp	Cys	Ala	Arg	Glu	Cys	
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30	ttc	acg	gag	cac	gac	gtg	aac	ttc	gcg	aac	cgg	ccg	ctg	ttc	ccg	tgc	336
	Phe	Thr	Glu	His	Asp	Val	Asn	Phe	Ala	Asn	Arg	Pro	Leu	Phe	Pro	Ser	
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	Met	Arg	Leu	Ala	Ser	Phe	Asp	Gly	Ala	Met	Leu	Ser	Val	Ser	Ser	Tyr	
			115					120					125				
40	ggc	ccg	tac	tgg	cgc	aac	ctg	cgc	cgc	gtc	gcc	gcc	gtg	cag	ctc	ctc	432
	Gly	Pro	Tyr	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Ala	Val	Gln	Leu	Leu	
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	Gly	Val	Ala	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	
				180					185					190			
15	agc	gtg	ctc	atg	gag	acc	atc	gcg	cac	acc	aag	acg	tcc	cgc	gcc	gag	624
	Ser	Val	Leu	Met	Glu	Thr	Ile	Ala	His	Thr	Lys	Thr	Ser	Arg	Ala	Glu	
				195				200					205				
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	Ala	Asp	Ala	Asn	Ser	Asp	Met	Ser	Thr	Glu	Ala	His	Glu	Phe	Lys	Gln	
		210					215					220					
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	225					230					235					240	
30	tac	ctg	ccg	gtg	ctg	cgc	tgg	ttc	gac	gtg	ttc	ggc	gtg	agg	aac	aag	768
	Tyr	Leu	Pro	Val	Leu	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys	
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	Asp	Gly	Glu	Arg	Arg	Arg	Leu	Asp	Ala	Gly	Asp	Glu	Ser	Glu	Ser	Lys	
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45	agc	atg	att	gcg	gtg	ctg	ctc	act	ctg	cag	aag	tcc	gag	cca	gag	gtc	912
	Ser	Met	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Ser	Glu	Pro	Glu	Val	
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50	tac	act	gac	act	gtg	atc	act	gct	ctt	tgc	gcg	aac	cta	ttc	ggc	gcc	960
	Tyr	Thr	Asp	Thr	Val	Ile	Thr	Ala	Leu	Cys	Ala	Asn	Leu	Phe	Gly	Ala	
	305					310					315					320	
55	gga	acg	gag	acc	acg	tcc	acc	acg	acg	gaa	tgg	gcc	atg	tca	ctg	ctg	1008
	Gly	Thr	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu	
					325					330					335		
60	ctg	aac	cac	cgg	gag	gcg	ctc	aag	aag	gcg	cag	gcc	gag	atc	gac	gcg	1056
	Leu	Asn	His	Arg	Glu	Ala	Leu	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala	
				340					345					350			
65	gcg	gtg	ggc	gcc	tcc	cgc	ctg	gtg	acc	gcg	gac	gac	gtg	ccc	cac	ctc	1104
	Ala	Val	Gly	Ala	Ser	Arg	Leu	Val	Thr	Ala	Asp	Asp	Val	Pro	His	Leu	
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	Thr	Tyr	Leu	Gln	Cys	Ile	Val	Asp	Glu	Thr	Leu	Arg	Leu	His	Pro	Ala	
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	Ala Pro Leu Leu Leu Pro His Glu Ser Ala Ala Asp Cys Thr Val Gly	
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5	ggc tac gac gtg ccg cgc ggc acg atg ctg ctg gtc aac gtg cac gcg	1248
	Gly Tyr Asp Val Pro Arg Gly Thr Met Leu Leu Val Asn Val His Ala	
	405 410 415	
10	gtc cac agg gac ccc gcg gtg tgg gag gac ccg gac agg ttc gtg ccg	1296
	Val His Arg Asp Pro Ala Val Trp Glu Asp Pro Asp Arg Phe Val Pro	
	420 425 430	
15	gag cgg ttc gag ggc gcc ggc ggc aag gcc gag ggg cgc ctg ctg atg	1344
	Glu Arg Phe Glu Gly Ala Gly Gly Lys Ala Glu Gly Arg Leu Leu Met	
	435 440 445	
20	ccg ttc ggg atg ggg cgg cgc aag tgc ccc ggg gag acg ctc gcg ctg	1392
	Pro Phe Gly Met Gly Arg Arg Lys Cys Pro Gly Glu Thr Leu Ala Leu	
	450 455 460	
25	cgg acc gtc ggg ctg gtg ctc gcc acg ctg ctc cag tgc ttc gac tgg	1440
	Arg Thr Val Gly Leu Val Leu Ala Thr Leu Leu Gln Cys Phe Asp Trp	
	465 470 475 480	
30	gac acg gtt gat gga gct cag gtt gac atg aag gct agc ggc ggg ctg	1488
	Asp Thr Val Asp Gly Ala Gln Val Asp Met Lys Ala Ser Gly Gly Leu	
	485 490 495	
35	acc atg ccc cgg gcc gtc ccg ttg gag gcc atg tgc agg ccg cgt aca	1536
	Thr Met Pro Arg Ala Val Pro Leu Glu Ala Met Cys Arg Pro Arg Thr	
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	Ala Lys Gly Ser Arg Arg Arg Leu Pro Pro Ser Pro Pro Ala Ile Pro	
	35 40 45	
	Phe Leu Gly His Leu His Leu Val Lys Ala Pro Phe His Gly Ala Leu	
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	Ala Arg Leu Ala Ala Arg His Gly Pro Val Phe Ser Met Arg Leu Gly	
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	Thr Arg Arg Ala Val Val Val Ser Ser Pro Asp Cys Ala Arg Glu Cys	
	85 90 95	

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	Met	Arg	Leu	Ala	Ser	Phe	Asp	Gly	Ala	Met	Leu	Ser	Val	Ser	Ser	Tyr
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	Arg	Ala	Met	Val	Arg	Arg	Met	Asp	Arg	Ala	Ala	Ala	Ala	Gly	Gly	Gly
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	Tyr	Leu	Pro	Val	Leu	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys
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	Gly	Thr	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu
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		355					360						365			
	Thr	Tyr	Leu	Gln	Cys	Ile	Val	Asp	Glu	Thr	Leu	Arg	Leu	His	Pro	Ala
		370				375						380				
35	Ala	Pro	Leu	Leu	Leu	Pro	His	Glu	Ser	Ala	Ala	Asp	Cys	Thr	Val	Gly
	385				390						395					400
	Gly	Tyr	Asp	Val	Pro	Arg	Gly	Thr	Met	Leu	Leu	Val	Asn	Val	His	Ala
				405						410					415	
	Val	His	Arg	Asp	Pro	Ala	Val	Trp	Glu	Asp	Pro	Asp	Arg	Phe	Val	Pro
			420						425					430		
40	Glu	Arg	Phe	Glu	Gly	Ala	Gly	Gly	Lys	Ala	Glu	Gly	Arg	Leu	Leu	Met
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<223> Black Mexican Sweet (BMS) maize line ORF for Nsf1

<221> CDS

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15	ctc cac tac ctc ctg ggc cgg cgg gcc ggc ggc gag ggc aag gcc aag	96
	Leu His Tyr Leu Leu Gly Arg Arg Ala Gly Gly Glu Gly Lys Ala Lys	
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20	gcc aag ggc tcg cgg cgg cgg ctc ccg ccg agc cct ccg gcg atc ccg	144
	Ala Lys Gly Ser Arg Arg Arg Leu Pro Pro Ser Pro Pro Ala Ile Pro	
	35 40 45	
25	ttc ctg ggc cac ctc cac ctc gtc aag gcc ccg ttc cac ggg gcg ctg	192
	Phe Leu Gly His Leu His Leu Val Lys Ala Pro Phe His Gly Ala Leu	
	50 55 60	
30	gcc cgc ctc gcg gcg cgc cac ggc ccg gtg ttc tcc atg cgc ctg ggg	240
	Ala Arg Leu Ala Ala Arg His Gly Pro Val Phe Ser Met Arg Leu Gly	
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	Thr Arg Arg Ala Val Val Val Ser Ser Pro Asp Cys Ala Arg Glu Cys	
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	Phe Thr Glu His Asp Val Asn Phe Ala Asn Arg Pro Leu Phe Pro Ser	
	100 105 110	
45	atg cgg ctg gcg tcc ttc gac ggc gcc atg ctc tcc gtg tcc agc tac	384
	Met Arg Leu Ala Ser Phe Asp Gly Ala Met Leu Ser Val Ser Ser Tyr	
	115 120 125	
50	ggc ccg tac tgg cgc aac ctg cgc cgc gtc gcc gcc gtg cag ctc ctc	432
	Gly Pro Tyr Trp Arg Asn Leu Arg Arg Val Ala Ala Val Gln Leu Leu	
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55	tcc gcg cac cgc gtc ggg tgc atg gcc ccc gcc atc gaa gcg cag gtg	480
	Ser Ala His Arg Val Gly Cys Met Ala Pro Ala Ile Glu Ala Gln Val	
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	Arg Ala Met Val Arg Arg Met Asp Arg Ala Ala Ala Ala Gly Gly Gly	
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65	ggc gtc gcg cgc gtc cag ctc aag cgg cgg ctg ttc gag ctc tcc ctc	576
	Gly Val Ala Arg Val Gln Leu Lys Arg Arg Leu Phe Glu Leu Ser Leu	
	180 185 190	
70	agc gtg ctc atg gag acc atc gcg cac acc aag acg tcc cgc gcc gag	624
	Ser Val Leu Met Glu Thr Ile Ala His Thr Lys Thr Ser Arg Ala Glu	
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		Tyr	Leu	Pro	Val	Leu	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys	
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		Ile	Leu	Asp	Ala	Val	Gly	Arg	Arg	Asp	Ala	Phe	Leu	Gly	Arg	Leu	Ile	
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		Asp	Gly	Glu	Arg	Arg	Arg	Leu	Asp	Ala	Gly	Asp	Glu	Ser	Glu	Ser	Lys	
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		Ser	Met	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Ser	Glu	Pro	Glu	Val	
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		Leu	Asn	His	Arg	Glu	Ala	Leu	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala	
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		Val	His	Arg	Asp	Pro	Ala	Val	Trp	Glu	Asp	Pro	Asp	Arg	Phe	Val	Pro	
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		Glu	Arg	Phe	Glu	Gly	Ala	Gly	Gly	Lys	Ala	Glu	Gly	Arg	Leu	Leu	Met	
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	Arg Thr Val Gly Leu Val Leu Ala Thr Leu Leu Gln Cys Phe Asp Trp	
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10	gac acg gtt gat gga gct cag gtt gac atg aag gct agc ggc ggg ctg	1488
	Asp Thr Val Asp Gly Ala Gln Val Asp Met Lys Ala Ser Gly Gly Leu	
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15	acc atg ccc cgg gcc gtc ccg ttg gag gcc atg tgc agg ccg cgt aca	1536
	Thr Met Pro Arg Ala Val Pro Leu Glu Ala Met Cys Arg Pro Arg Thr	
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	Ala Lys Gly Ser Arg Arg Arg Leu Pro Pro Ser Pro Pro Ala Ile Pro	
	35 40 45	
	Phe Leu Gly His Leu His Leu Val Lys Ala Pro Phe His Gly Ala Leu	
	50 55 60	
40	Ala Arg Leu Ala Ala Arg His Gly Pro Val Phe Ser Met Arg Leu Gly	
	65 70 75 80	
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	85 90 95	
	Phe Thr Glu His Asp Val Asn Phe Ala Asn Arg Pro Leu Phe Pro Ser	
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	Gly Pro Tyr Trp Arg Asn Leu Arg Arg Val Ala Ala Val Gln Leu Leu	
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	Ser Ala His Arg Val Gly Cys Met Ala Pro Ala Ile Glu Ala Gln Val	
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	Gly Val Ala Arg Val Gln Leu Lys Arg Arg Leu Phe Glu Leu Ser Leu	
	180 185 190	
55	Ser Val Leu Met Glu Thr Ile Ala His Thr Lys Thr Ser Arg Ala Glu	
	195 200 205	
	Ala Asp Ala Asp Ser Asp Met Ser Thr Glu Ala His Glu Phe Lys Gln	
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Ile Val Asp Glu Leu Val Pro Tyr Ile Gly Thr Ala Asn Arg Trp Asp
 225 230 235 240
 Tyr Leu Pro Val Leu Arg Trp Phe Asp Val Phe Gly Val Arg Asn Lys
 245 250 255
 5 Ile Leu Asp Ala Val Gly Arg Arg Asp Ala Phe Leu Gly Arg Leu Ile
 260 265 270
 Asp Gly Glu Arg Arg Arg Leu Asp Ala Gly Asp Glu Ser Glu Ser Lys
 275 280 285
 10 Ser Met Ile Ala Val Leu Leu Thr Leu Gln Lys Ser Glu Pro Glu Val
 290 295 300
 Tyr Thr Asp Thr Val Ile Thr Ala Leu Cys Ala Asn Leu Phe Gly Ala
 305 310 315 320
 Gly Thr Glu Thr Thr Ser Thr Thr Thr Glu Trp Ala Met Ser Leu Leu
 325 330 335
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 340 345 350
 Ala Val Gly Thr Ser Arg Leu Val Thr Ala Asp Asp Val Pro His Leu
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 Thr Tyr Leu Gln Cys Ile Val Asp Glu Thr Leu Arg Leu His Pro Ala
 370 375 380
 20 Ala Pro Leu Leu Leu Pro His Glu Ser Ala Ala Asp Cys Thr Val Gly
 385 390 395 400
 Gly Tyr Asp Val Pro Arg Gly Thr Met Leu Leu Val Asn Val His Ala
 405 410 415
 Val His Arg Asp Pro Ala Val Trp Glu Asp Pro Asp Arg Phe Val Pro
 420 425 430
 25 Glu Arg Phe Glu Gly Ala Gly Gly Lys Ala Glu Gly Arg Leu Leu Met
 435 440 445
 Pro Phe Gly Met Gly Arg Arg Lys Cys Pro Gly Glu Thr Leu Ala Leu
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 Arg Thr Val Gly Leu Val Leu Ala Thr Leu Leu Gln Cys Phe Asp Trp
 465 470 475 480
 30 Asp Thr Val Asp Gly Ala Gln Val Asp Met Lys Ala Ser Gly Gly Leu
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<221> CDS

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 Leu His Tyr Leu Leu Gly Arg Arg Ala Gly Gly Glu Gly Lys Ala Lys
 20 25 30

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5	ttc	ctg	ggc	cac	ctc	cac	ctc	gtc	aag	gcc	ccg	ttc	cac	ggg	gcg	ctg	192
	Phe	Leu	Gly	His	Leu	His	Leu	Val	Lys	Ala	Pro	Phe	His	Gly	Ala	Leu	
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10	gcc	cgc	ctc	gcg	gcg	cgc	cac	ggc	ccg	gtg	ttc	tcc	atg	cgc	ctg	ggg	240
	Ala	Arg	Leu	Ala	Ala	Arg	His	Gly	Pro	Val	Phe	Ser	Met	Arg	Leu	Gly	
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15	acc	cgg	cgc	gcc	gtg	gtc	gtg	tcg	tcg	ccg	gac	tgc	gcc	agg	gag	tgc	288
	Thr	Arg	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Asp	Cys	Ala	Arg	Glu	Cys	
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	Phe	Thr	Glu	His	Asp	Val	Asn	Phe	Ala	Asn	Arg	Pro	Leu	Phe	Pro	Ser	
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	Met	Arg	Leu	Ala	Ser	Phe	Asp	Gly	Ala	Met	Leu	Ser	Val	Ser	Ser	Tyr	
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	Gly	Pro	Tyr	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Ala	Val	Gln	Leu	Leu	
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	Ser	Ala	His	Arg	Val	Gly	Cys	Met	Ala	Pro	Ala	Ile	Glu	Ala	Gln	Val	
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	Arg	Ala	Met	Val	Arg	Arg	Met	Asp	Arg	Ala	Ala	Ala	Ala	Gly	Gly	Gly	
					165					170					175		
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40	agc	gtg	ctc	atg	gag	acc	atc	gcg	cac	acc	aag	acg	tcc	cgc	gcc	gag	624
	Ser	Val	Leu	Met	Glu	Thr	Ile	Ala	His	Thr	Lys	Thr	Ser	Arg	Ala	Glu	
			195					200					205				
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	Ala	Asp	Ala	Asp	Ser	Asp	Met	Ser	Thr	Glu	Ala	His	Glu	Phe	Lys	Gln	
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	225					230					235					240	
50	tac	ctg	ccg	gtg	ctg	cgc	tgg	ttc	gac	gtg	ttc	ggc	gtg	agg	aac	aag	768
	Tyr	Leu	Pro	Val	Leu	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys	
					245					250					255		
55	atc	ctc	gac	gcc	gtg	ggc	aca	agg	gac	gcg	ttc	ctg	ggg	cgg	ctc	atc	816
	Ile	Leu	Asp	Ala	Val	Gly	Thr	Arg	Asp	Ala	Phe	Leu	Gly	Arg	Leu	Ile	
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		gac	ggg	gag	cgg	cgg	agg	ctg	gac	gct	ggc	gac	gag	agc	gaa	agt	aag	864
		Asp	Gly	Glu	Arg	Arg	Arg	Leu	Asp	Ala	Gly	Asp	Glu	Ser	Glu	Ser	Lys	
			275						280					285				
5		agc	atg	att	gcg	gtg	ctg	ctc	act	ctg	cag	aag	tcc	gag	cca	gag	gtc	912
		Ser	Met	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Ser	Glu	Pro	Glu	Val	
			290					295					300					
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		Tyr	Thr	Asp	Thr	Val	Ile	Thr	Ala	Leu	Cys	Ala	Asn	Leu	Phe	Gly	Ala	
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15		gga	acg	gag	acc	acg	tcc	acc	acg	acg	gaa	tgg	gcc	atg	tca	ctg	ctg	1008
		Gly	Thr	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu	
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		ctg	aac	cac	cgg	gag	gcg	ctc	aag	aag	gcg	cag	gcc	gag	atc	gac	gcg	1056
		Leu	Asn	His	Arg	Glu	Ala	Leu	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala	
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20		gcg	gtg	ggc	acc	tcc	cgc	ctg	gtg	acc	gcg	gac	gac	gtg	ccc	cac	ctc	1104
		Ala	Val	Gly	Thr	Ser	Arg	Leu	Val	Thr	Ala	Asp	Asp	Val	Pro	His	Leu	
				355					360					365				
25		acc	tac	ctg	cag	tgc	atc	gtc	gac	gag	acg	ctg	cgc	ctg	cac	ccg	gcc	1152
		Thr	Tyr	Leu	Gln	Cys	Ile	Val	Asp	Glu	Thr	Leu	Arg	Leu	His	Pro	Ala	
			370					375					380					
30		gcg	ccg	ctg	ctg	ctg	ccg	cac	gag	tcc	gcc	gcg	gac	tgc	acg	gtc	ggc	1200
		Ala	Pro	Leu	Leu	Leu	Pro	His	Glu	Ser	Ala	Ala	Asp	Cys	Thr	Val	Gly	
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		ggc	tac	gac	gtg	ccg	cgc	ggc	acg	atg	ctg	ctg	gtc	aac	gtg	cac	gcg	1248
		Gly	Tyr	Asp	Val	Pro	Arg	Gly	Thr	Met	Leu	Leu	Val	Asn	Val	His	Ala	
					405						410					415		
35		gtc	cac	agg	gac	ccc	gcg	gtg	tgg	gag	gac	ccg	gac	agg	ttc	gtg	ccg	1296
		Val	His	Arg	Asp	Pro	Ala	Val	Trp	Glu	Asp	Pro	Asp	Arg	Phe	Val	Pro	
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		Glu	Arg	Phe	Glu	Gly	Ala	Gly	Gly	Lys	Ala	Glu	Gly	Arg	Leu	Leu	Met	
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		ccg	ttc	ggg	atg	ggg	cgg	cgc	aag	tgc	ccc	ggg	gag	acg	ctc	gcg	ctg	1392
		Pro	Phe	Gly	Met	Gly	Arg	Arg	Lys	Cys	Pro	Gly	Glu	Thr	Leu	Ala	Leu	
			450					455					460					
45		cgg	acc	gtc	ggg	ctg	gtg	ctc	gcc	acg	ctg	ctc	cag	tgc	ttc	gac	tgg	1440
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50		gac	acg	gtt	gat	gga	gct	cag	gtt	gac	atg	aag	gct	agc	ggc	ggg	ctg	1488
		Asp	Thr	Val	Asp	Gly	Ala	Gln	Val	Asp	Met	Lys	Ala	Ser	Gly	Gly	Leu	
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55		acc	atg	ccc	cgg	gcc	gtc	ccg	ttg	gag	gcc	atg	tgc	agg	ccg	cgt	aca	1536
		Thr	Met	Pro	Arg	Ala	Val	Pro	Leu	Glu	Ala	Met	Cys	Arg	Pro	Arg	Thr	
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		gct	atg	cgt	ggt	gtt	ctt	aag	agg	ctc	tga							1566
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 <223> F2 maize line Nsf1 peptide

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Ala	Lys	Gly	Ser	Arg	Arg	Arg	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Ile	Pro
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Phe	Leu	Gly	His	Leu	His	Leu	Val	Lys	Ala	Pro	Phe	His	Gly	Ala	Leu
	50					55					60				
Ala	Arg	Leu	Ala	Ala	Arg	His	Gly	Pro	Val	Phe	Ser	Met	Arg	Leu	Gly
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Thr	Arg	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Asp	Cys	Ala	Arg	Glu	Cys
				85					90					95	
Phe	Thr	Glu	His	Asp	Val	Asn	Phe	Ala	Asn	Arg	Pro	Leu	Phe	Pro	Ser
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Met	Arg	Leu	Ala	Ser	Phe	Asp	Gly	Ala	Met	Leu	Ser	Val	Ser	Ser	Tyr
		115					120					125			
Gly	Pro	Tyr	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Ala	Val	Gln	Leu	Leu
	130					135						140			
Ser	Ala	His	Arg	Val	Gly	Cys	Met	Ala	Pro	Ala	Ile	Glu	Ala	Gln	Val
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Arg	Ala	Met	Val	Arg	Arg	Met	Asp	Arg	Ala	Ala	Ala	Ala	Gly	Gly	Gly
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			180					185					190		
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Ala	Asp	Ala	Asp	Ser	Asp	Met	Ser	Thr	Glu	Ala	His	Glu	Phe	Lys	Gln
	210					215					220				
Ile	Val	Asp	Glu	Leu	Val	Pro	Tyr	Ile	Gly	Thr	Ala	Asn	Arg	Trp	Asp
225					230					235					240
Tyr	Leu	Pro	Val	Leu	Arg	Trp	Phe	Asp	Val	Phe	Gly	Val	Arg	Asn	Lys
				245					250					255	
Ile	Leu	Asp	Ala	Val	Gly	Thr	Arg	Asp	Ala	Phe	Leu	Gly	Arg	Leu	Ile
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Asp	Gly	Glu	Arg	Arg	Arg	Leu	Asp	Ala	Gly	Asp	Glu	Ser	Glu	Ser	Lys
		275					280					285			
Ser	Met	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Ser	Glu	Pro	Glu	Val
	290					295					300				
Tyr	Thr	Asp	Thr	Val	Ile	Thr	Ala	Leu	Cys	Ala	Asn	Leu	Phe	Gly	Ala
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Gly	Thr	Glu	Thr	Thr	Ser	Thr	Thr	Thr	Glu	Trp	Ala	Met	Ser	Leu	Leu
				325					330					335	
Leu	Asn	His	Arg	Glu	Ala	Leu	Lys	Lys	Ala	Gln	Ala	Glu	Ile	Asp	Ala
			340					345					350		

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 355 360 365
 Thr Tyr Leu Gln Cys Ile Val Asp Glu Thr Leu Arg Leu His Pro Ala
 370 375 380
 5 Ala Pro Leu Leu Leu Pro His Glu Ser Ala Ala Asp Cys Thr Val Gly
 385 390 395 400
 Gly Tyr Asp Val Pro Arg Gly Thr Met Leu Leu Val Asn Val His Ala
 405 410 415
 10 Val His Arg Asp Pro Ala Val Trp Glu Asp Pro Asp Arg Phe Val Pro
 420 425 430
 Glu Arg Phe Glu Gly Ala Gly Gly Lys Ala Glu Gly Arg Leu Leu Met
 435 440 445
 Pro Phe Gly Met Gly Arg Arg Lys Cys Pro Gly Glu Thr Leu Ala Leu
 450 455 460
 15 Arg Thr Val Gly Leu Val Leu Ala Thr Leu Leu Gln Cys Phe Asp Trp
 465 470 475 480
 Asp Thr Val Asp Gly Ala Gln Val Asp Met Lys Ala Ser Gly Gly Leu
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 Thr Met Pro Arg Ala Val Pro Leu Glu Ala Met Cys Arg Pro Arg Thr
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<212> DNA

<213> Zea mays

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<223> GA209 maize line ORF for Nsf1

<221> CDS

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<400> 23

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	1				5					10					15		
5	ctc	cac	tac	ctc	ctg	ggc	cgg	cgg	gcc	ggc	gtc	gag	ggc	aag	gcc	aag	96
	Leu	His	Tyr	Leu	Leu	Gly	Arg	Arg	Ala	Gly	Val	Glu	Gly	Lys	Ala	Lys	
				20					25					30			
10	ggc	tcg	cgg	cgg	cgg	ctc	ccg	ccg	agc	cct	ccg	gcg	atc	ccg	ttc	ctg	144
	Gly	Ser	Arg	Arg	Arg	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Ile	Pro	Phe	Leu	
			35					40					45				
15	ggc	cac	ctc	cac	ctc	gtc	aag	gcc	ccg	ttc	cac	ggg	gcg	ctg	gcc	cgc	192
	Gly	His	Leu	His	Leu	Val	Lys	Ala	Pro	Phe	His	Gly	Ala	Leu	Ala	Arg	
		50					55					60					
20	ctc	gcg	gcg	cgc	cac	ggc	ccg	gtg	ttc	tcc	atg	cgc	ctg	ggg	acc	cgg	240
	Leu	Ala	Ala	Arg	His	Gly	Pro	Val	Phe	Ser	Met	Arg	Leu	Gly	Thr	Arg	
	65					70				75						80	
25	cgc	gcc	gtg	gtc	gtg	tcg	tcg	ccg	gac	tgc	gcc	agg	gag	tgc	ttc	acg	288
	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Asp	Cys	Ala	Arg	Glu	Cys	Phe	Thr	
					85					90					95		
30																	
35																	
40																	
45																	
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		gag	cac	gac	gtg	aac	ttc	gcg	aac	cgg	ccg	ctg	ttc	ccg	tcg	atg	cgg	336
		Glu	His	Asp	Val	Asn	Phe	Ala	Asn	Arg	Pro	Leu	Phe	Pro	Ser	Met	Arg	
					100					105					110			
5		ctg	gcg	tcc	ttc	gac	ggc	gcc	atg	ctc	tcc	gtg	tcc	agc	tac	ggc	ccg	384
		Leu	Ala	Ser	Phe	Asp	Gly	Ala	Met	Leu	Ser	Val	Ser	Ser	Tyr	Gly	Pro	
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		tac	tgg	cgc	aac	ctg	cgc	cgc	gtc	gcc	gcc	gtg	cag	ctc	ctc	tcc	gcg	432
10		Tyr	Trp	Arg	Asn	Leu	Arg	Arg	Val	Ala	Ala	Val	Gln	Leu	Leu	Ser	Ala	
			130					135					140					
		cac	cgc	gtc	ggg	tgc	atg	gcc	ccc	gcc	atc	gaa	gcg	cag	gtg	cgc	gcc	480
15		His	Arg	Val	Gly	Cys	Met	Ala	Pro	Ala	Ile	Glu	Ala	Gln	Val	Arg	Ala	
		145					150					155					160	
		atg	gtg	cgg	agg	atg	gac	cgc	gcc	gcc	gcg	gcc	ggc	ggc	ggc	ggc	gtc	528
		Met	Val	Arg	Arg	Met	Asp	Arg	Ala	Ala	Ala	Ala	Gly	Gly	Gly	Gly	Val	
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		Ala	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	Ser	Val	
					180					185					190			
		ctc	atg	gaa	acc	atc	gcg	cac	acc	aag	acg	tcc	cgc	gcc	gag	gcc	gac	624
25		Leu	Met	Glu	Thr	Ile	Ala	His	Thr	Lys	Thr	Ser	Arg	Ala	Glu	Ala	Asp	
				195					200					205				
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			210					215					220					
		aac	gag	ctc	gtg	ccg	tac	atc	ggc	acg	gcc	aac	tgc	tgg	gac	tac	ctg	720
		Asn	Glu	Leu	Val	Pro	Tyr	Ile	Gly	Thr	Ala	Asn	Cys	Trp	Asp	Tyr	Leu	
		225					230				235					240		
35		ccg	gtg	ctg	cgc	tgg	ttc	gac	gtg	ttc	ggc	gtg	agg	aac	aag	atc	ctc	768
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40		Asp	Ala	Val	Gly	Arg	Arg	Asp	Ala	Phe	Leu	Gly	Arg	Leu	Ile	Asp	Gly	
					260					265					270			
		gag	cgg	cgc	agg	ctg	gac	gct	ggc	gac	gag	agc	gaa	agt	aag	agc	atg	864
		Glu	Arg	Arg	Arg	Leu	Asp	Ala	Gly	Asp	Glu	Ser	Glu	Ser	Lys	Ser	Met	
				275					280					285				
45		att	gcg	gtg	ctg	ctc	act	ctg	cag	aag	tcc	gag	cca	gag	gtc	tac	act	912
		Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Ser	Glu	Pro	Glu	Val	Tyr	Thr	
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		gac	act	gtg	atc	act	gct	ctg	tta	gca	act	ctg	tcg	gct	agg	gca	cga	960
50		Asp	Thr	Val	Ile	Thr	Ala	Leu	Leu	Ala	Thr	Leu	Ser	Ala	Arg	Ala	Arg	
		305					310					315					320	
		aca	aca	gtc	gct	aga	gat	gta	gag	aac	gct	aga	ggc	gtg	gag	aac	agg	1008
55		Thr	Thr	Val	Ala	Arg	Asp	Val	Glu	Asn	Ala	Arg	Gly	Val	Glu	Asn	Arg	
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 Lys Ile *

1057

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 <222> (0)...(0)
 <223> GA209 maize line Nsf1 peptide

30 <400> 24

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5	Gly	Ser	Arg	Arg	Arg	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Ile	Pro	Phe	Leu
			35				40						45			
	Gly	His	Leu	His	Leu	Val	Lys	Ala	Pro	Phe	His	Gly	Ala	Leu	Ala	Arg
		50					55					60				
	Leu	Ala	Ala	Arg	His	Gly	Pro	Val	Phe	Ser	Met	Arg	Leu	Gly	Thr	Arg
10	65					70					75					80
	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Asp	Cys	Ala	Arg	Glu	Cys	Phe	Thr
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	Glu	His	Asp	Val	Asn	Phe	Ala	Asn	Arg	Pro	Leu	Phe	Pro	Ser	Met	Arg
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	Leu	Ala	Ser	Phe	Asp	Gly	Ala	Met	Leu	Ser	Val	Ser	Ser	Tyr	Gly	Pro
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	His	Arg	Val	Gly	Cys	Met	Ala	Pro	Ala	Ile	Glu	Ala	Gln	Val	Arg	Ala
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					165					170					175	
	Ala	Arg	Val	Gln	Leu	Lys	Arg	Arg	Leu	Phe	Glu	Leu	Ser	Leu	Ser	Val
				180					185					190		
	Leu	Met	Glu	Thr	Ile	Ala	His	Thr	Lys	Thr	Ser	Arg	Ala	Glu	Ala	Asp
			195					200					205			
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		210					215					220				
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	225					230					235					240
30																
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	Asp	Ala	Val	Gly	Arg	Arg	Asp	Ala	Phe	Leu	Gly	Arg	Leu	Ile	Asp	Gly
				260					265					270		
35	Glu	Arg	Arg	Arg	Leu	Asp	Ala	Gly	Asp	Glu	Ser	Glu	Ser	Lys	Ser	Met
			275					280					285			
	Ile	Ala	Val	Leu	Leu	Thr	Leu	Gln	Lys	Ser	Glu	Pro	Glu	Val	Tyr	Thr
		290					295					300				
	Asp	Thr	Val	Ile	Thr	Ala	Leu	Leu	Ala	Thr	Leu	Ser	Ala	Arg	Ala	Arg
	305					310					315					320
40	Thr	Thr	Val	Ala	Arg	Asp	Val	Glu	Asn	Ala	Arg	Gly	Val	Glu	Asn	Arg
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45 <211> 1932

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<213> Zea mays

<220>

50 <221> misc_feature

<222> (0)...(0)

<223> W703A maize line ORF for Nsf1

<221> CDS

55 <222> (1)...(843)

<400> 25

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	Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Asp	Cys	Ala	Arg	Glu	Cys	Phe	Thr	
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	Glu	His	Asp	Val	Asn	Phe	Ala	Asn	Arg	Pro	Leu	Phe	Pro	Ser	Met	Arg	
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	Leu	Ala	Ser	Phe	Asp	Gly	Ala	Met	Leu	Ser	Val	Ser	Ser	Tyr	Gly	Pro	
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40																	
45																	
50																	
55																	

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			20					25					30		
Ser	Ser	Arg	Arg	Arg	Leu	Pro	Pro	Ser	Pro	Pro	Ala	Ile	Pro	Phe	Leu
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Gly	His	Leu	His	Leu	Val	Lys	Ala	Pro	Phe	His	Ala	Ala	Leu	Ala	Arg
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Arg	Ala	Val	Val	Val	Ser	Ser	Pro	Asp	Cys	Ala	Arg	Glu	Cys	Phe	Thr
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Glu	His	Asp	Val	Asn	Phe	Ala	Asn	Arg	Pro	Leu	Phe	Pro	Ser	Met	Arg
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Val	Asp	Arg	Gly	Pro	Arg	Val	Gln	Ala	Xaa	Arg	Gln	Arg	Ala	Arg	Ala
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Val	His	Arg	Arg	Gly	Gln	Pro	Leu	Gly	Leu	Pro	Ala	Gly	Ala	Ala	Leu
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Val	Arg	Arg	Val	Arg	Arg	Glu	Glu	Gln	Asp	Pro	Arg	Arg	Arg	Gly	Gln
				245					250					255	
Lys	Gly	Arg	Val	Pro	Glu	Ala	Ala	His	Arg	Arg	Gly	Ala	Ala	Glu	Ala
			260					265					270		
Gly	Arg	Trp	Arg	Arg	Gln	Arg	Lys								
		275					280								

Claims

1. Use of a DNA construct comprising a polynucleotide comprising:

(a) the nucleotide sequence of SEQ ID No. 1;
 (b) a nucleotide sequence encoding a polypeptide capable of conferring resistance to at least one herbicide to a plant, wherein said herbicide is a member of a class of herbicides selected from:

- (i) the ALS-inhibiting class;
- (ii) the pigment synthesis-inhibiting class;
- (iii) the PPO-inhibiting class;
- (iv) the PS II-inhibiting class; and

(v) the synthetic auxin class

wherein the polypeptide has an amino acid sequence of at least 85%, at least 90%, or at least 95% identity, when compared to SEQ ID No. 2 based on the Needleman-Wunsch alignment algorithm, or

(c) a complement of the nucleotide sequence of (a) or (b), wherein the complement and the nucleotide sequence consist of the same number of nucleotides and are 100% complementary;

operably linked to at least one regulatory sequence; to confer or enhance resistance to at least one herbicide to a plant, or to at least two, three, four or five herbicides, wherein said herbicides are selected from a class of herbicides selected from:

- (a) the ALS-inhibiting class;
- (b) the pigment synthesis-inhibiting class;
- (c) the PPO-inhibiting class;
- (d) the PS II-inhibiting class; and
- (e) the synthetic auxin class.

2. The use of claim 1, wherein the herbicide is selected from:

ALS-inhibiting herbicides selected from:

- (a) nicosulfuron;
- (b) rimsulfuron;
- (c) primisulfuron;
- (d) imazethapyr;
- (e) chlorsulfuron;
- (f) chlorimuron ethyl;
- (g) triasulfuron;
- (h) flumetsulam; and
- (i) imazaquin;

pigment synthesis-inhibiting herbicides selected from:

- (a) isoxaflutole;
- (b) topramezone;
- (c) sulcatrione; and
- (d) tembotrione;

PPO-inhibiting herbicides selected from:

- (a) acifluofen;
- (b) flumioxan; and
- (c) sulfentrazone;

PS II-inhibiting herbicides selected from:

- (a) diuron;
- (b) linuron;
- (c) bentazon; and
- (d) chlorotoluron; and

the herbicide dicamba.

3. The use of claim 1, wherein said plant is a monocot or a dicot.

4. The use of claim 3, wherein said monocot is selected from maize, wheat, barley, oats, switchgrass, millet, sugarcane, sorghum, and rice; or wherein said dicot is selected from soybean, canola, potato, cotton, alfalfa and sunflower.

5. The use of claim 1, wherein said plant further comprises a second herbicide resistance gene or a gene encoding an insecticidal polypeptide.

6. The use of claim 5, wherein said plant further comprises a gene encoding a polypeptide with glyphosate N-acetyltransferase activity or a second herbicide resistance gene encoding a polypeptide conferring tolerance to ALS inhibitors.

7. The use of claim 1, wherein the plant is a soybean plant that also exhibits soybean cyst nematode resistance and has been bred from germplasm selected from:

- (a) Peking;
- (b) PI88788;
- (c) PI89772;
- (d) PI90763;
- (e) PI209332;
- (f) PI404189A;
- (g) PI437654;
- (h) PI438489B;
- (i) PI467312;
- (j) PI468916;
- (k) Hartwig;
- (l) J87-233; and
- (m) progeny derived from sources (a) through (l).

Patentansprüche

1. Verwendung eines DNA-Konstrukts, umfassend ein Polynukleotid, umfassend:

- (a) die Nukleotidsequenz von SEQ ID Nr. 1;
- (b) eine Nukleotidsequenz, die für ein Polypeptid codiert, das dazu fähig ist, einer Pflanze Resistenz gegen zumindest ein Herbizid zu verleihen, wobei das Herbizid ein Mitglied einer Klasse von Herbiziden ist, die ausgewählt ist aus:

- (i) der ALS-hemmenden Klasse;
- (ii) der Pigmentsynthese-hemmenden Klasse;
- (iii) der PPO-hemmenden Klasse;
- (iv) der PS-II-hemmenden Klasse; und
- (v) der Klasse synthetischen Auxins,

wobei das Polypeptid eine Aminosäuresequenz von mindestens 85%, mindestens 90% oder mindestens 95% Identität, bei Vergleich mit SEQ ID Nr. 2, aufweist bezogen auf den Needleman-Wunsch-Alignment-Algorithmus, oder

- (c) ein Komplement der Nukleotidsequenz aus (a) oder (b), wobei das Komplement und die Nukleotidsequenz aus der gleichen Anzahl von Nukleotiden bestehen und zu 100% komplementär sind;

funktionell verknüpft mit zumindest einer regulatorischen Sequenz;

um einer Pflanze Resistenz gegen zumindest ein Herbizid zu verleihen oder ihre Resistenz dagegen zu stärken, oder gegen zumindest zwei, drei, vier oder fünf Herbizide, wobei die Herbizide aus einer Klasse von Herbiziden ausgewählt sind, die ausgewählt ist aus:

- (a) der ALS-hemmenden Klasse;
- (b) der Pigmentsynthese-hemmenden Klasse;
- (c) der PPO-hemmenden Klasse;
- (d) der PS-II-hemmenden Klasse; und
- (e) der Klasse synthetischen Auxins.

2. Verwendung nach Anspruch 1, wobei das Herbizid ausgewählt ist aus:

ALS-hemmenden Herbiziden, ausgewählt aus:

- 5 (a) Nicosulfuron;
(b) Rimsulfuron;
(c) Primisulfuron;
(d) Imazethapyr;
(e) Chlorsulfuron;
(f) Chlorimuronethyl;
10 (g) Triasulfuron;
(h) Flumetsulam; und
(i) Imazaquin;

Pigmentsynthese-hemmenden Herbiziden, ausgewählt aus:

- 15 (a) Isoxaflutol;
(b) Topramezon;
(c) Sulcatrion; und
(d) Tembotrion;

20 PPO-hemmenden Herbiziden, ausgewählt aus:

- (a) Acifluofen;
(b) Flumioxan; und
25 (c) Sulfentrazone;

PS-II-hemmenden Herbiziden, ausgewählt aus:

- (a) Diuron;
(b) Linuron;
30 (c) Bentazon; und
(d) Chlortoluron; und

dem Herbizid Dicamba.

- 35 **3.** Verwendung nach Anspruch 1, wobei die Pflanze eine Monokotyledone oder eine Dikotyledone ist.
- 4.** Verwendung nach Anspruch 3, wobei die Monokotyledone aus Mais, Weizen, Gerste, Hafer, Rutenhirse, Millethirse, Zuckerrohr, Sorghumhirse und Reis ausgewählt ist; oder wobei die Dikotyledone aus Sojabohne, Raps, Kartoffel, Baumwolle, Luzerne und Sonnenblume ausgewählt ist.
- 40 **5.** Verwendung nach Anspruch 1, wobei die Pflanze weiterhin ein zweites Herbizidresistenzgen oder ein Gen umfasst, das für ein insektizides Polypeptid codiert.
- 6.** Verwendung nach Anspruch 5, wobei die Pflanze weiterhin ein Gen umfasst, das für ein Polypeptid mit Glyphosat-N-Acetyltransferase-Aktivität codiert, oder ein zweites Herbizidresistenzgen, das für ein Toleranz gegenüber ALS-Hemmern verleihendes Polypeptid codiert.
- 45 **7.** Verwendung nach Anspruch 1, wobei die Pflanze eine Sojabohnenpflanze ist, die weiterhin Sojabohnen-Zysten-Nematoden-Resistenz aufweist und aus Keimplasma gezüchtet worden ist, das ausgewählt ist aus:

- 50 (a) Peking;
(b) PI88788;
(c) PI89772;
(d) PI90763;
55 (e) PI209332;
(f) PI404189A;
(g) PI437654;
(h) PI438489B;

- (i) PI467312;
- (j) PI468916;
- (k) Hartwig;
- (l) J87-233; und
- (m) von Quellen (a) bis (1) stammenden Nachkommen.

Revendications

1. Utilisation d'une construction d'ADN comprenant un polynucléotide comprenant:

- (a) la séquence nucléotidique de SEQ ID N° 1;
- (b) une séquence nucléotidique codant pour un polypeptide apte à conférer une résistance à au moins un herbicide à une plante, ledit herbicide étant un membre d'une classe d'herbicides choisie parmi:

- (i) la classe inhibant l'ALS;
- (ii) la classe inhibant la synthèse des pigments;
- (iii) la classe inhibant la PPO;
- (iv) la classe inhibant le PS II; et
- (v) la classe des auxines synthétiques

dans laquelle le polypeptide a une séquence d'acides aminés ayant au moins 85%, au moins 90%, ou au moins 95% d'identité, avec SEQ ID N° 2 d'après l'algorithme d'alignement de Needleman-Wunsch, ou

- (c) une séquence complémentaire de la séquence nucléotidique de (a) ou (b), la séquence complémentaire et la séquence nucléotidique étant constituées du même nombre de nucléotides et étant à 100% complémentaires;

fonctionnellement liée à au moins une séquence régulatrice;

pour conférer à une plante ou accroître chez une plante la résistance à au moins un herbicide, ou à au moins deux, trois, quatre ou cinq herbicides, lesdits herbicides étant choisis dans une classe d'herbicides choisie parmi:

- (a) la classe inhibant l'ALS;
- (b) la classe inhibant la synthèse des pigments;
- (c) la classe inhibant la PPO;
- (d) la classe inhibant le PS II; et
- (e) la classe des auxines synthétiques.

2. Utilisation de la revendication 1, dans laquelle l'herbicide est choisi parmi:

les herbicides inhibant l'ALS choisis parmi:

- (a) nicosulfuron;
- (b) rimsulfuron;
- (c) primisulfuron;
- (d) imazéthapyr;
- (e) chlorsulfuron;
- (f) chlorimuron-éthyl;
- (g) triasulfuron;
- (h) flumetsulam; et
- (i) imazaquine;

les herbicides inhibant la synthèse des pigments, choisis parmi:

- (a) isoxaflutole;
- (b) topramézone;
- (c) sulcatrione; et
- (d) tembotrione;

les herbicides inhibant la PPO choisis parmi:

- (a) acifluofène;
- (b) flumioxan; et
- (c) sulfentrazone;

5 les herbicides inhibant le PS II choisis parmi:

- (a) diuron;
- (b) linuron;
- (c) bentazon; et
- (d) chlorotoluron; et

10

l'herbicide dicamba.

3. Utilisation de la revendication 1, dans laquelle ladite plante est une monocotylédone ou une dicotylédone.

15

4. Utilisation de la revendication 3, dans laquelle ladite monocotylédone est choisie parmi le maïs, le blé, l'orge, l'avoine, le panic érigé, le millet, la canne à sucre, le sorgho, et le riz; ou dans laquelle ladite dicotylédone est choisie parmi le soja, le canola, la pomme de terre, le coton, la luzerne et le tournesol.

20

5. Utilisation de la revendication 1, dans laquelle ladite plante comprend en outre un second gène de résistance à un herbicide ou un gène codant pour un polypeptide insecticide.

6. Utilisation de la revendication 5, dans laquelle ladite plante comprend en outre un gène codant pour un polypeptide ayant une activité de glyphosate N-acétyltransférase ou un second gène de résistance à un herbicide codant pour un polypeptide conférant une tolérance aux inhibiteurs de l'ALS.

25

7. Utilisation de la revendication 1, dans laquelle la plante est un plant de soja qui présente également une résistance au nématode à kystes du soja et a été produit à partir d'un germoplasme choisi parmi:

30

- (a) Peking;
- (b) PI88788;
- (c) PI89772;
- (d) PI90763;
- (e) PI209332;
- (f) PI404189A;
- (g) PI437654;
- (h) PI438489B;
- (i) PI467312;
- (j) PI468916;
- (k) Hartwig;
- (l) J87-233; et
- (m) la descendance issue des sources (a) à (l).

35

40

45

50

55

Figure 1a

Symbol comparison table: blosum62.cmp CompCheck: 1102

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BAD27507	SEQ ID NO: 9
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rice_nsf_hom	SEQ ID NO: 4
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XP_469849	SEQ ID NO: 11
nsf_peptide	SEQ ID NO: 2

//

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aak38081	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
BAD27508	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
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BAD27506	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
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XP_469849	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
nsf_peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~

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rice_nsf_hom	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
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XP_469849	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
nsf_peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~

Figure 1b

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rice_nsf_hom	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
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XP_469849	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
nsf_peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
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BAD27508	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
aak38079	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
BAD27507	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
BAD27506	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
rice_nsf_hom	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
xp_469850	hhkkmpsstt	tktttprsda	wivsarsdpf	hllleaqaapl	gikadalsqi
XP_469849	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
nsf_peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	201				250
XP_469851	~~~~~	~~~~~m	DKAY.IAvfs	iAiLFLLvdY	frcrrrrgsg
XP_469852	~~~~~	~~~~~m	vKAY.IAifs	iAvLLLi..h	flfrrr...g
aak38080	~~~~~	~~~~~m	DKAY.IAiLS	cAflFLvH.Y	vLGk..vsdg
aak38081	~~~~~	~~~~~m	DKAY.IAiLS	cAflflvH.Y	vLGk..vsdg
BAD27508	~~~~~	~~~~~m	DKAY.IAiLS	cAflFLvH.Y	vLGk..vsdg
aak38079	~~~~~	~~~~~m	DKAY.IAiLS	sAflFLvH.Y	vLGk..vsdg
BAD27507	~~~~~	~~~~~m	DKAY.IAiLS	cAflFLvH.Y	vLGk..vshg
BAD27506	~~~~~	~~~~~m	DKAY.IAiLS	cAflFLvH.Y	vLGk..vshg
rice_nsf_hom	~~~~~	~~~~~m	DnAYiIAiLS	vAiLFLLHyY	LLgr..gngg
xp_469850	aavhqshrnt	shirelsam	DnAYiIAiLS	vAiLFLLHyY	LLgr..gngg
XP_469849	~~~~~	~~~~~m	DKAY.IAvfs	iviLFLLvdY	Lrrl..rggg
nsf_peptide	~~~~~	~~~~~M	DKAY.IAALS	AAALFLLH.Y	LLGRRAGGEG
	251				300
XP_469851	snngenkgml	qLPPSPPAIP	FfGHLHLidk	PIHaALsRLA	eRHGPVFS1R
XP_469852	rsng....m	pLPPSPPAIP	FfGHLHLidk	PfHaALsRLA	eRHGPVFS1R
aak38080	.rrgK.kgav	qLPPSPPAvP	FLGHLHLVdk	PiHatmcRLA	AR1GPVFS1R
aak38081	.rrgK.kgav	qLPPSPPAvP	FLGHLHLVdk	PiHatmcRLA	AR1GPVFS1R
BAD27508	.rrgK.kgav	qLPPSPPAIP	FiGHLHLVek	PiHatmcRLA	AR1GPVFS1R
aak38079	.rrgK.kgav	qLPPSPPAvP	FLGHLHLVek	PiHatmcRLA	AR1GPVFS1R
BAD27507	.rrgK.kgav	qLPPSPPAIP	FiGHLHLVek	PiHatmcRLA	AR1GPVFS1R
BAD27506	.rrgK.kgav	qLPPSPPAIP	FiGHLHLVek	PiHatmcRLA	AR1GPVFS1R
rice_nsf_hom	aA.....	RLPPgPPAvP	ilGHLHLVkk	PmHatmsRLA	eRyGPVFS1R
xp_469850	aA.....	RLPPgPPAvP	ilGHLHLVkk	PmHatmsRLA	eRyGPVFS1R
XP_469849	tsngK.nkgm	RLPPgLPavP	iiGHLHLVkk	PmHatLsRLA	ARHGPVFS1R
nsf_peptide	KAKAK.GSRR	RLPPSPPAIP	FLGHLHLVKA	PFHGALARLA	ARHGPVFSMR

Figure 1c

	301				350
XP_469851	LGsRnAVVVS	SPeCARECFT	dnDVcFANRP	qFPSqmpAtF	ygAgfgfanY
XP_469852	LGsRnAVVVS	SPeCARECFT	dnDVcFANRP	rFPSqmLatF	nGtsLgsanY
aak38080	LGsRRRAVVVS	SseCARECFT	EHDVtFANRP	kFPSqLLvSF	nGtaLvtSSY
aak38081	LGsRRRAVVVS	SseCARECFT	EHDVtFANRP	kFPSqLLvSF	nGtaLvtSSY
BAD27508	LGsRRRAVVVp	SseCARECFT	EHDVtFANRP	kFPSqLLASF	nGtaLvtSSY
aak38079	LGsRRRAVVVS	SseCARECFT	EHDVtFANRP	kFPSqLLvSF	nGtaLvtSSY
BAD27507	LGsRRRAVVVS	SseCARECFT	EHDVtFANRP	ssrrklLASF	nGtaLvtSSY
BAD27506	LGsRRRAVVVS	SseCARECFT	EHDVtFANRP	kFPSqLLASF	nGtaLvtSPSY
rice_nsf_hom	LGsRRRAVVVS	SPgCARECFT	EHDVtFANRP	rFeSqLLvSF	nGaaLataSY
xp_469850	LGsRRRAVVVS	SPgCARECFT	EHDVtFANRP	rFeSqLLvSF	nGaaLataSY
XP_469849	LGsRRRAVVVS	SPgCARECFT	EHDVtFANRP	rFeSqLLmSF	DGtaLamaSY
nsf_peptide	LGTRRAVVVS	SPDCARECFT	EHDVNEFANRP	LFPSMRLASF	DGAMLSVSSY
	351				400
XP_469851	GahWRNLRRi	AtVhLLSAhR	VrgMAgvvsg	eiRpMVqRM	RAAAAagvGV
XP_469852	GPhWRNLRRi	AtVhLLSShR	VsgMsiglIs	QaRhMVRRM	RAAtAsaaGV
aak38080	GPhWRNLRRV	AtVQLLSAhr	VaCMsgvIaA	eVRAMaRRlf	hAteAspdGa
aak38081	GPhWRNLRRV	AtVQLLSAhr	VaCMsgvIaA	eVRAMaRRlf	hAAeAspdGa
BAD27508	GPhWRNLRRV	AtVQLLSAhr	VaCMsgvIaA	eVRAMaRRlf	hAAeAspdGa
aak38079	GPhWRNLRRV	AtVQLLSAhr	VtCMsgvIaA	eVRAMaRRlf	hAAeAspdGa
BAD27507	GPhWRNLRRV	AtVQLLSAhr	VaCMsgvIaA	eVRAMaRRlf	hAAeAspdGa
BAD27506	GPhWRNLRRV	AtVQLLSAhr	VaCMsgvIaA	eVRAMaRRlf	hAAeAspgGa
rice_nsf_hom	GahWRNLRRi	vAVQLLSAhr	VGlMsiglIag	eVRAMVRRM	RAAAAAspaGa
xp_469850	GahWRNLRRi	vAVQLLSAhr	VGlMsiglIag	eVRAMVRRM	RAAAAAspaGa
XP_469849	GPhWRNLRRV	AAVQLLSAhr	VGlMsiglIag	eVRAMVRslc	R....rpaaa
nsf_peptide	GPYWRNLRRV	AAVQLLSAHR	VGCMAPIEA	QVRAMVRRMD	RAAAAAGGGV
	401				450
XP_469851	ARVQLKRRLF	ELSLSVLMEa	IAqTKtRpe	adDADtDMSv	EAqEFKvD
XP_469852	ARVQLKRRLF	ELSLSVLMEa	IAqSKtRrE	apDADtDMSm	EAqElrhvD
aak38080	ARVQLKRRLF	ELSLSVLMET	IAqTKatRse	.ADADtDMSv	EAqEFKevVD
aak38081	ARVQLKRRLF	ELSLSVLMET	IAqTKatRse	.ADADtDMSv	EAqEFKevVD
BAD27508	ARVQLKRRLF	ELSLSVLMET	IAqTKatRse	.ADADtDMSv	EAqEFKevVD
aak38079	ARVQLKRRLF	ELSLSVLMET	IAqTKatRse	.ADADtDMSl	EAqEFKevVD
BAD27507	tRVQLKRRLF	ELSLSVLMET	IAqTKatRse	.ADADtDMSv	EAqEFKevVD
BAD27506	ARVQLKRgpf	ELSLSVLMET	IAqTKatRse	.aDADtDMSv	EAqEFKevVD
rice_nsf_hom	ARiQLKRRLF	EvSLSVLMET	IAHTKatRpe	.tDpDtDMSv	EAqEFKQvVD
xp_469850	ARiQLKRRLF	EvSLSVLMET	IAHTKatRpe	.tDpDtDMSv	EAqEFKQvVD
XP_469849	ApVQLKRRLF	ELSLSVLMET	IAqSKatRpe	ttDtDtDMSm	EAqEyKQvVe
nsf_peptide	ARVQLKRRLF	ELSLSVLMET	IAHTKTSRAE	.ADADSDMST	EAHEFKQIVD
	451				500
XP_469851	ELnPlIGaAN	lWDYLPaLRv	FDVlGVkrKI	atlanRRDAF	vrRLIDaERq
XP_469852	ELnPlIGaAN	lWDYLPaLRW	FDVFGVkrKI	vaAVnRRnAF	mrRLIDaERq
aak38080	kLiPhlGaAN	mWDYLPVmrW	FDVFGVRNKI	LhAVsRRDAF	LrRLIDaERR
aak38081	kLiPhlGaAN	mWDYLPVmrW	FDVFGVRNKI	LhAVsRRDAF	LrRLIDaERR
BAD27508	kLiPhlGaAN	mWDYLPVmrW	FDVFGVRNKI	LhAVsRRDAF	LrRLIDaERR
aak38079	kLiPhlGaAN	mWDYLPVmrW	FDVFGVRsKI	LhAVsRRDAF	LrRLInaERR
BAD27507	kLiPhlGaAN	mWDYLPVmrW	FDVFGVRNKI	LhAVsRRDAF	LrRLIDaERR
BAD27506	kpiPhlGaAN	mWDYLPVmrW	FDVFGVRNKI	LhAVsRRDAF	LrRLIDaERR
rice_nsf_hom	EiiPhlGaAN	lWDYLPaLRW	FDVFGVRrKI	LaAVsRRDAF	LrRLIDaERR
xp_469850	EiiPhlGaAN	lWDYLPaLRW	FDVFGVRrKI	LaAVsRRDAF	LrRLIDaERR
XP_469849	EilerIGTgN	lCDYLPaLRW	FDVFGVRNrI	LaAVsRRDAF	LrRLIyaaRw
nsf_peptide	ELVPYIGTAN	RWDYLPVLRW	FDVFGVRNKI	LDAVGRRDAF	LGRLIDGERR

Figure 1d

	501				550
XP_469851	Rmdng.vDgG	DdgEkKSvIs	VLLsLQKtEP	EVYkDivIvn	LCAaLFaAGT
XP_469852	RmdndvDgG	DdgEkKSMIs	VLLTLQKtqP	EVYTDtIImt	LCaPlFGAGT
aak38080	RL....aDgG	sdgdkKSMIA	VLLTLQKtEP	kVYTDtMITA	LCANLFGAGT
aak38081	RL....aDgG	sdgdkKSMIA	VLLTLQKtEP	kVYTDtMITA	LCANLFGAGT
BAD27508	RL....aDgG	sdgdkKSMIA	VLLTLQKtEP	kVYTDtMITA	LCANLFGAGT
aak38079	RL....aDgG	sdgdkKSMIA	VLLTLQKtEP	kVYTDtMITA	LCANLFGAGT
BAD27507	RL....aDgG	sdgdkKSMIA	VLLTLQKtEP	kVYTDtMITA	LCANLFGAGT
BAD27506	RL....aDgG	sdgdkKSMIA	VLLTLQKtEP	kVYTDtMITA	LCANLFGAGT
rice_nsf_hom	RL.....DdG	DEgEkKSMIA	VLLTLQKtEP	EVYTDnmITA	LtANLFGAGT
xp_469850	RL.....DdG	DEgEkKSMIA	VLLTLQKtEP	eVYTDnmITA	LtANLFGAGT
XP_469849	Rm.....Dd.	.gEkKSMIA	VLLTLQKtqP	EVYTDnmITA	LCsNLlGAGT
nsf_peptide	RL.....DAG	DESESKSMIA	VLLTLQKSEP	EVYTDtVITA	LCANLFGAGT
	551				600
XP_469851	ETTAmTiEWA	MSLLLNHpki	LKKakAEIDA	sVGnSRLing	DDmPHLsYLQ
XP_469852	ETTSTTiEWA	MSLLLNHpEi	LKKAQAEIDm	sVGnSRLisv	VDVhrLgYLQ
aak38080	ETTSTTTEWA	MSLLLNHpAa	LKKAQAEIDA	sVGTSRLVsv	DDVPSLaYLQ
aak38081	ETTSTTTEWA	MSLLLNHpAa	LKKAQAEIDA	sVGTSRLVsv	DDVPSLaYLQ
BAD27508	ETTSTTTEWA	MSLLLNHpAa	LKKAQAEIDA	sVGTSRLVsv	DDVPSLaYLQ
aak38079	ETTSTTTEWA	MSLLLNHpAa	LKKAQAEIDA	sVGTSRLVsv	DDVPSLaYLQ
BAD27507	ETTSTTTEWA	MSLLLNHpAa	LKKAQAEIDA	sVGTSRLVsv	DDVlsLaYLQ
BAD27506	ETTSTTTErA	MSLLLNHpAa	LKKAQAEIDA	sVGTSRLVsv	DDmPSLaYLQ
rice_nsf_hom	ETTSTTSEWA	MSLLLNHpdt	LKKAQAEIDA	sVGnSRLiTA	DDVtrLgYLQ
xp_469850	ETTSTTSEWA	MSLLLNHpdt	LKKAQAEIDA	sVGnSRLiTA	DDVtrLgYLQ
XP_469849	ETTSTTiEWA	MSLLLNHpEt	LKKAQAEIDA	sVGnSRLiTA	DDVPriTYLQ
nsf_peptide	ETTSTTTEWA	MSLLLNHREA	LKKAQAEIDA	AVGTSRLVTA	DDVPHLTYLQ
	601				650
XP_469851	CIinETLRly	PvAPLLlPHE	SsADCKvNGY	hiPsGTMLLV	NViAiqRDPm
XP_469852	CIinETLRmy	PAAPLLLPHE	SsADCKvGGY	hiPsGaMLLV	NVAiqRDPv
aak38080	CIVseTLRLy	PAAPLLLPHE	SsADCKvGGY	nVPadTMLiV	NayAiHRDPA
aak38081	CIVnETLRly	PAAPLLLPHE	SsADCKvGGY	nVPadTMLiV	NayAiHRDPA
BAD27508	CIVnETLRly	PAAPLLLPHE	SsADCKvGGY	nVPadTMLiV	NayAiHRDPA
aak38079	CIVseTLRLy	PAAPLLLPHE	SsADCKvGGY	nVPadTMLiV	NayAiHRDPA
BAD27507	CIVseTLRLy	PAAPLLLPHE	SsADCKvGGY	nVPadTMLiV	NayAiHRDPA
BAD27506	CIVnETLRly	PAAPLLLPHE	SsADCKvGGY	nVPadTMLiV	NayAiHRDPA
rice_nsf_hom	CIVreTLRLy	PAAPmLLPHE	SsADCKvGGY	niPRGsMLLi	NayAiHRDPA
xp_469850	CIVreTLRLy	PAAPmLLPHE	SsADCKvGGY	niPRGsMLLi	NayAiHRDPA
XP_469849	CIVreTLRLy	PAAPmLiPHE	SsADCEvGGY	sVPRGTMLLV	NayAiHRDPA
nsf_peptide	CIVDETLRLH	PAAPLLLPHE	SAADCTVGGY	DVPRGTMLLV	NVHAVHRDPA
	651				700
XP_469851	VWkePneFkP	ERFE..nGes	EGLfmiPFGM	GRRKCPGETm	ALqTiGLVLg
XP_469852	iWkePseFkP	ERFE..nGrf	EGLfmiPFGM	GRRrCPGEmL	ALqTiGLVLg
aak38080	aWEdPleFrP	ERFE..dGKA	EGLfmiPFGM	GRRrCPGETL	ALRTiGmVLA
aak38081	aWEhPlvFrP	ERFE..dGKA	EGLfmiPFGM	GRRrCPGETL	ALRTiGmVLA
BAD27508	aWEhPleFrP	ERFE..dGKA	EGLfmiPFgV	GRRrCPGETL	ALRTismVLA
aak38079	aWEdPleFkP	ERFE..dGKA	EGLfmiPFGM	GRRrCPGETL	ALRTiGmVLA
BAD27507	aWEhPleFrP	ERFE..dGKA	EGLfmiPFGM	GRRrCPGETL	ALRTiGmVLA
BAD27506	aWEhPleFrP	ERFE..dGKA	EGLfmiPFGM	GRRrCPGETL	ALRTiGmVLA
rice_nsf_hom	VWEePekFmP	ERFE..dGgc	dGnLLMPFGM	GRRrCPGETL	ALRTVGLVLg
xp_469850	VWEePekFmP	ERFE..dGgc	dGnLLMPFGM	GRRrCPGETL	ALRTVGLVLg
XP_469849	aWEePerFVP	ERFE..GGgc	dGnLSMPFGM	GRRrCPGETL	ALhTVGLVLg
nsf_peptide	VWEDPDRFVP	ERFEGAGGKA	EGRLLMFPGM	GRRKCPGETL	ALRTVGLVLA

Figure 1e

	701					750
XP_469851	aLiQCFDWD	r	VDGAeVDMtq	gsGLTnPRAV	PLEAMCkPRe	AMsdVfReLl
XP_469852	TmiQCFDWgr		VDdAmVDMtq	SnGLTslkvi	PLEAMCkPRe	AMcdVLRkfm
aak38080	TLvQCFDWep		VDGvkVDMte	gGGfTiPkAV	PLEAvCRPRa	vMRdVLqnL~
aak38081	TLvQCFDWep		VDGvnVDMte	gGGfTiPkAV	PLEAvCRPRa	vMRdVLqsi~
BAD27508	TLvQCFDWep		VDGvkVDMte	gGGfTiPkAV	PLEAvCRPRa	vMRdVLqnL~
aak38079	TLvQCFDWep		VDGvkVDMte	gGGfTiPkAV	PLEAvCRPRv	vMRdVLqnL~
BAD27507	TLvQCFDWep		VDGvkVDMte	gGGfTiPkAV	PLEAvCRPRt	vMRdVLqnL~
BAD27506	TLvQCFDWep		VDGvkVDMte	gGGfTiPkAV	PLEAvCRPRa	vMRdVLqnL~
rice_nsf_hom	TLiQCFDWer		VDGveVDMte	gGGLTiPkvv	PLEAMCRPRd	AMgGVLReLv
xp_469850	TLiQCFDWer		VDGveVDMte	gGGLTiPkvv	PLEAMCRPRd	AMgGVLReLv
XP_469849	TLiQCFDWer		VDGveVDMae	gGGLTMPkvV	PLEAvCRPRd	AMgGVLReL~
nsf_peptide	TLLQCFDWD	T	VDGAQVDMKA	SGGLTMPRAV	PLEAMCRPRt	AMRGVLKRL~

Figure 2a

Symbol comparison table: blosum62.cmp CompCheck: 1102GapWeight: 8
GapLengthWeight: 2F2_peptide = SEQ ID NO: 22
nsf_peptide = SEQ ID NO: 2
BMS_peptide = SEQ ID NO: 20
Q66_Peptide = SEQ ID NO: 18
GA209_Peptide = SEQ ID NO: 24
W703A_Peptide = SEQ ID NO: 26

	1				50
F2_peptide	MDKAYIAALS	AAALFLLHYL	LGRRAGGEGK	AKAKGSRRRL	PPSPPAIPFL
nsf_peptide	MDKAYIAALS	AAALFLLHYL	LGRRAGGEGK	AKAKGSRRRL	PPSPPAIPFL
BMS_peptide	MDKAYIAALS	AAALFLLHYL	LGRRAGGEGK	AKAKGSRRRL	PPSPPAIPFL
Q66_Peptide	MDKAYIAALS	AAALFLLHYL	LGRRAGGEGK	AKAKGSRRRL	PPSPPAIPFL
GA209_Peptide	MDKAYIAALS	AAALFLLHYL	LGRRAGVEG.	.KAKGSRRRL	PPSPPAIPFL
W703A_Peptide	MDKAYIAALS	AAALFLLHYL	LGRRAGVEG.	.KAKSSRRRL	PPSPPAIPFL

	51				100
F2_peptide	GHLHLVKAPF	HGALARLAAR	HGPVFMSRLG	TRRAVVVSSP	DCARECFTEH
nsf_peptide	GHLHLVKAPF	HGALARLAAR	HGPVFMSRLG	TRRAVVVSSP	DCARECFTEH
BMS_peptide	GHLHLVKAPF	HGALARLAAR	HGPVFMSRLG	TRRAVVVSSP	DCARECFTEH
Q66_Peptide	GHLHLVKAPF	HGALARLAAR	HGPVFMSRLG	TRRAVVVSSP	DCARECFTEH
GA209_Peptide	GHLHLVKAPF	HGALARLAAR	HGPVFMSRLG	TRRAVVVSSP	DCARECFTEH
W703A_Peptide	GHLHLVKAPF	HAALARLAAR	HGPVFMSRLG	TRRAVVVSSP	DCARECFTEH

	101				150
F2_peptide	DVNFANRPLF	PSMRLASFDG	AMLSVSSYGP	YWRNLRRVAA	VQLLSAHRVG
nsf_peptide	DVNFANRPLF	PSMRLASFDG	AMLSVSSYGP	YWRNLRRVAA	VQLLSAHRVG
BMS_peptide	DVNFANRPLF	PSMRLASFDG	AMLSVSSYGP	YWRNLRRVAA	VQLLSAHRVG
Q66_Peptide	DVNFANRPLF	PSMRLASFDG	AMLSVSSYGP	YWRNLRRVAA	VQLLSAHRVG
GA209_Peptide	DVNFANRPLF	PSMRLASFDG	AMLSVSSYGP	YWRNLRRVAA	VQLLSAHRVG
W703A_Peptide	DVNFANRPLF	PSMRLASFDG	AMLSVSSYGP	YWRNLRRVAA	VQLLSAHRVA

	151				200
F2_peptide	CMAPIEAQV	RAMVRRMDRA	AAAGGGGVAR	VQLKRRLFEL	SLSVLMETIA
nsf_peptide	CMAPIEAQV	RAMVRRMDRA	AAAGGGGVAR	VQLKRRLFEL	SLSVLMETIA
BMS_peptide	CMAPIEAQV	RAMVRRMDRA	AAAGGGGVAR	VQLKRRLFEL	SLSVLMETIA
Q66_Peptide	CMAPIEAQV	RAMVRRMDRA	AAAGGGGVAR	VQLKRRLFEL	SLSVLMETIA
GA209_Peptide	CMAPIEAQV	RAMVRRMDRA	AAAGGGGVAR	VQLKRRLFEL	SLSVLMETIA
W703A_Peptide	CMVPIEAQV	RAMVRRMDRA	AAAGGARRAR	PAQAAAVRAL	PQRAHGNHRA

	201				250
F2_peptide	HTKTSRAEAD	ADSDMSTEA.	HEFKQIVDEL	VPYIGTANRW	DYLPVLRWFD
nsf_peptide	HTKTSRAEAD	ADSDMSTEA.	HEFKQIVDEL	VPYIGTANRW	DYLPVLRWFD
BMS_peptide	HTKTSRAEAD	ADSDMSTEA.	HEFKQIVDEL	VPYIGTANRW	DYLPVLRWFD
Q66_Peptide	HTKTSRAEAD	ANSMDMSTEA.	HEFKQIVNEL	VPYIGTANRW	DYLPVLRWFD
GA209_Peptide	HTKTSRAEAD	ANSMDMSTEA.	HEFKQIVNEL	VPYIGTANCW	DYLPVLRWFD
W703A_Peptide	HQDVPRQLEX	VDRGPRVQAX	RQRARAVHRR	GQPLG.....	..LPAGAAL.

Figure 2b

	251				300
F2_peptide	VFGVRNKILD	AVGTRDAFLG	RLIDGERRR.	.LDAGDESES	KSMIAVLLTL
nsf_peptide	VFGVRNKILD	AVGRRDAFLG	RLIDGERRR.	.LDAGDESES	KSMIAVLLTL
BMS_peptide	VFGVRNKILD	AVGRR.AFLG	RLIDGERRR.	.LDAGDESES	KSMIAVLLTL
Q66_Peptide	VFGVRNKILD	AVGRRDAFLG	RLIDGERRR.	.LDAGDESES	KSMIAVLLTL
GA209_Peptide	VFGVRNKILD	AVGRRDAFLG	RLIDGERRR.	.LDAGDESES	KSMIAVLLTL
W703A_Peptide	VRRVRREEQD	P..RRRGQKG	RVPEAAHRRG	AAEAGRWRRO	RK~~~~~
	301				350
F2_peptide	QKSEPEVYTD	TVITALCANL	FGAGTETTST	TTEWAMSLLL	NHREALKKAQ
nsf_peptide	QKSEPEVYTD	TVITALCANL	FGAGTETTST	TTEWAMSLLL	NHREALKKAQ
BMS_peptide	QKSEPEVYTD	TVITALCANL	FGAGTETTST	TTEWAMSLLL	NHREALKKAQ
Q66_Peptide	QKSEPEVYTD	TVITALCANL	FGAGTETTST	TTEWAMSLLL	NHREALKKAQ
GA209_Peptide	QKSEPEVYTD	TVITALLATL	SARARTTVAR	DVENARG..V	ENRKI~~~~
W703A_Peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	351				400
F2_peptide	AEIDAAVGTS	RLVTADDVPH	LTYLQCIVDE	TLRLHPAAPL	LLPHESAADC
nsf_peptide	AEIDAAVGTS	RLVTADDVPH	LTYLQCIVDE	TLRLHPAAPL	LLPHESAADC
BMS_peptide	AEIDAAVGTS	RLVTADDVPH	LTYLQCIVDE	TLRLHPAAPL	LLPHESAADC
Q66_Peptide	AEIDAAVGTS	RLVTADDVPH	LTYLQCIVDE	TLRLHPAAPL	LLPHESAADC
GA209_Peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
W703A_Peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	401				450
F2_peptide	TVGGYDVPRG	TMLLVNVHAV	HRDPAVWEDP	DRFVPERFEG	AGGKAEGRLL
nsf_peptide	TVGGYDVPRG	TMLLVNVHAV	HRDPAVWEDP	DRFVPERFEG	AGGKAEGRLL
BMS_peptide	TVGGYDVPRG	TMLLVNVHAV	HRDPAVWEDP	DRFVPERFEG	AGGKAEGRLL
Q66_Peptide	TVGGYDVPRG	TMLLVNVHAV	HRDPAVWEDP	DRFVPERFEG	AGGKAEGRLL
GA209_Peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
W703A_Peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	451				500
F2_peptide	MEFGMGRRKC	PGETLALRTV	GLVLATLLQC	FDWDTVDGAQ	VDMKASGGLT
nsf_peptide	MEFGMGRRKC	PGETLALRTV	GLVLATLLQC	FDWDTVDGAQ	VDMKASGGLT
BMS_peptide	MEFGMGRRKC	PGETLALRTV	GLVLATLLQC	FDWDTVDGAQ	VDMKASGGLT
Q66_Peptide	MEFGMGRRKC	PGETLALRTV	GLVLATLLQC	FDWDTVDGAQ	VDMKASGGLT
GA209_Peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
W703A_Peptide	~~~~~	~~~~~	~~~~~	~~~~~	~~~~~
	501		524		
F2_peptide	MPRAVPLEAM	CRPRTAMRGV	LKRL		
nsf_peptide	MPRAVPLEAM	CRPRTAMRGV	LKRL		
BMS_peptide	MPRAVPLEAM	CRPRTAMRGV	LKRL		
Q66_Peptide	MPRAVPLEAM	CRPRTAMRGV	LKRL		
GA209_Peptide	~~~~~	~~~~~	~~~~~		
W703A_Peptide	~~~~~	~~~~~	~~~~~		

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